Medical
RC
271
.C5
C265
v.67
no.3

SERIAL

1983

cancer treatment reports

MAY 19 1983
MEDICAL LIBRARY

March 1983
Volume 67
Number 3
Pages 209-325

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20.3160:67/3

507-H-9

cancer treatment reports

The University of Michigan Medical Library

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***
Division of Cancer Treatment, Bruce A. Chabner, ***Director***
Scientific Information Branch, Susan Molloy Hubbard, ***Chief***

Editorial Board

John S. Macdonald, ***Editor-In-Chief***

Associate Editors

Joseph Aisner
Lewis Aronow
Desmond Carney
Richard L. Cysyk
William D. DeWys
Ross C. Donehower
Daniel C. Ihde
Allen S. Lichter
Robert W. Makuch
David P. Pistenmaa
David G. Poplack
Frederick P. Smith

Advisory Board

Gianni Bonadonna
George P. Canellos
Richard H. Creech
Harvey M. Golomb
Robert L. Goodman
Stephen B. Howell
Randall K. Johnson
Irwin H. Krakoff
Brigid G. Leventhal
Robert B. Livingston
Albert F. LoBuglio
William L. McGuire
Franco M. Muggia
Carol S. Portlock
Sydney E. Salmon

Editorial Staff

Jean Griffin Baum, ***Managing Editor***
Dee Kittner LeRoy, ***Assistant Managing Editor***

Editors

Elaine Price Beck
Anne McCarthy

Assistants

Florence Barnes
Elaine Brown
Jana Johnston
Barbara Lineberry

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20814 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears in the second page of every issue.

UNIVERSITY OF MICHIGAN LIBRARIES
MAY 5 1983

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

DEPOSITED BY THE UNITED STATES OF AMERICA

INSTRUCTIONS TO AUTHORS

Title Page
Include complete title of the manuscript and the full name, affiliation, and address of each author on the title page. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use only standard abbreviations; otherwise use full words.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, chapter title, book title, city and name of publisher, date of publication, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers published in meeting proceedings should include author; title of paper; title of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs should be clear, glossy prints. If original art work is submitted, it should be done in black ink for good reproduction. Please send 2 copies of each print. Photocopies reproduce poorly. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge; 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior or specified author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

Contents

Cancer Treatment Reports
Volume 67, Number 3
March 1983

Current Controversies in Cancer Management

209 Is Chest Radiotherapy Necessary in Any or All Patients With Small Cell Carcinoma of the Lung? Yes. **R. W. Byhardt and J. D. Cox.** The Medical College of Wisconsin Affiliated Hospitals, Milwaukee

217 Is Thoracic Radiation Therapy Necessary for Patients With Limited-Stage Small Cell Lung Cancer? No. **M. H. Cohen.** Veterans Administration Medical Center, Washington, DC

Special Feature

223 Strategies for the Prevention of Infection in the Myelosuppressed or Immunosuppressed Cancer Patient. **P. A. Pizzo and S. C. Schimpff.** National Cancer Institute, Bethesda, Maryland

Clinical and Preclinical Studies

235 Ip Cisplatin in Patients With Malignant Ascites: Pharmacokinetic Evaluation and Comparison With the Iv Route. **E. S. Casper, D. P. Kelsen, N. W. Alcock, and J. L. Lewis, Jr.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

239 Phase I Study of MVE-2 Therapy in Human Cancer. **A. Rios, M. Rosenblum, M. Powell, and E. Hersh.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

245 Pharmacokinetics of Therapeutic Doses of Isotopically Labeled Platinum Antitumor Agents in the Mouse and Rat. **A. B. Robins and M. O. Leach.** Royal Marsden Hospital, Sutton, Surrey, England

253 Effect of Nutritional Status on the Hepatobiliary Excretion of Methotrexate in the Rat. **V. B. Grossie, Jr, and T. L. Loo.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

259 Effect of Pretreatment With Phenobarbital or SKF 525A on the Toxicity and Antitumor Activity of Lomustine. **D. W. Siemann.** University of Rochester Cancer Center, New York

267 Biochemical Pharmacology and Toxicology of Formycin Alone and in Combination With 2′-Deoxycoformycin (Pentostatin). **E. N. Spremulli, G. W. Crabtree, D. L. Dexter, S. H. Chu, D. M. Farineau, L. Y. Ghoda, D. L. McGowan, I. Diamond, R. E. Parks, Jr, and P. Calabresi.** Roger Williams General Hospital and Brown University, Providence, Rhode Island

275 Antitumor Activity of Tetrahydro-2-furanyl- and Tetrahydro-2-pyranyl-glucans Obtained by Chemical Modification of (1→3)-β-D-Glucan From *Alcaligenes faecalis* var. *myxogenes* IFO 13140 and Its Lower Molecular Weight Glucans. **T. Sasaki, N. Abiko, H. Uchida, M. Tanaka, K. Nitta, Y. Yoshioka, and H. Nomura.** National Cancer Center Research Institute, Tokyo, Japan

BRIEF REPORTS

281 Intermittent High-Dose Aclarubicin in Patients With Advanced Cancer: A Phase I Study With Special Reference to Cardiac Toxicity. **K. Aabo, S. A. Mortensen, T. Skovsgaard, and E. Gymoese.** Finsen Institute, Copenhagen, Denmark

283 Dialyzability of Methyl-GAG. **B. Jensen, R. D. Williams, T. Ohnuma, and J. Roboz.** Veterans Administration Hospital, and the University of California School of Medicine, San Francisco

285 Phase II Evaluation of Anguidine in Central Nervous System Tumors: A Southwest Oncology Group Study. **J. W. Goodwin, R. H. Bottomley, C. B. Vaughn, J. Frank, and R. P. Pugh.** Southwest Oncology Group

287 Cyclophosphamide, Doxorubicin, Vincristine, and Dexamethasone in Primary Lymphoma of the Brain: A Case Report. **D. J. Stewart, N. Russell, E. A. Atack, A. Quarrington, and L. Stolbach.** Ontario Cancer Treatment and Research Foundation Clinic, Ottawa, and the University of Ottawa, Ontario, Canada

293 Phase II Study of Vinblastine in Small Cell Carcinoma of the Lung. **S. Sörenson, K. Østerlind, P. Dombernowsky, and H. H. Hansen.** Renströmska Hospital, Göteborg, Sweden

297 Use of High-Dose Oral Methotrexate Sequenced at 24 Hours With 5-FU: A Clinical Toxicity Study. **C. Benz, M. Silverberg, and E. Cadman.** Yale University School of Medicine, New Haven, Connecticut

301 Mitomycin-Associated Lung Disease. **S. R. Gunstream, J. J. Seidenfeld, R. E. Sobonya, and L. J. McMahon.** Veterans Administration Medical Center, and Arizona Health Sciences Center, Tucson

CLINICAL TRIALS SUMMARIES

305 PCNU in Advanced Breast Cancer. **S. Martino, B. A. Samal, and A. Singhakowinta.** Wayne State University, Detroit, Michigan

307 Phase II Trial of Bisantrene in Advanced Colorectal Carcinoma. **T. Ahmed, N. E. Kemeny, R. A. Michaelson, and H. D. Harper.** Memorial Sloan-Kettering Cancer Center, New York, New York

309 Phase II Study of AMSA in Refractory Testicular Cancer. **S. D. Williams, P. Duncan, and L. H. Einhorn.** Indiana University Medical Center, Indianapolis

311 Phase II Trial of ICRF-187 in Non-Small Cell Lung Cancer. **R. B. Natale, R. H. Wheeler, M. K. Liepman, A. Sauder, and L. Bricker.** University of Michigan, Ann Arbor, and Ann Arbor Veterans Administration Medical Center, Michigan

LETTERS

315 Responder Versus Nonresponder Comparisons: Daunorubicin Plus Prednisone in Treatment of Acute Nonlymphocytic Leukemia. **N. Mantel.** The American University, Bethesda, Maryland

316 Phase II Study of Vindesine in Patients With Hodgkin's Disease, Lymphoma, and Breast Cancer. **N. Gad-El-Mawla, M. R. Hamza, M. El-Serafi, A. El-Khoudary, M. Aboul-Einein, and S. El-Demerdash.** National Cancer Institute, Cairo, Egypt

317 Raynaud's Phenomenon and Cryoglobulinemia During Chemotherapy for Testicular Carcinoma. **P. R. Dunlop and P. M. Hendy-Ibbs.** Weston Park Hospital, Sheffield, England

318 Dantrolene and Potentially Fatal Hyperthermia Secondary to L-Asparaginase. **W. A. Smithson, G. A. Gronert, and K. K. Moss.** Mayo Clinic, Rochester, Minnesota

319 Effect of Intermittent and Continuous Administration of L-Cysteine Hydrochloride on L1210 Mouse Leukemia. **T. Knight, E. C. Besa, R. R. Joseph, and S. I. Baskin.** The Medical College of Pennsylvania, Philadelphia

320 Amsacrine (AMSA) in the Treatment of Lymphoma. **R. P. Warrell, Jr.** Memorial Sloan-Kettering Cancer Center, New York, New York

323 ANNOUNCEMENTS

Is Chest Radiotherapy Necessary in Any or All Patients With Small Cell Carcinoma of the Lung? Yes.[1]

Roger W. Byhardt and James D. Cox[2,3,*]

Concepts of treatment for small cell carcinoma of the lung (SCCL) have evolved steadily over the past decade. Initially, SCCL was viewed simply as a variant of "lung cancer," but it displayed a virulent behavior when treated with conventional approaches. Median survival times of untreated patients were 12 weeks for limited disease (LD) and 5 weeks for extensive disease (ED) (1,2). Surgery alone for selected patients with resectable cancer provided median survival times up to 6 months, but most patients still demonstrated early dissemination. In the Medical Research Council comparison of radiation therapy and surgery for patients with LD, radical radiotherapy increased median survival to 11 months, with 30% of patients surviving 1 year and 10% surviving 2 years, despite the use of a variety of radiation doses, some of which were inadequate by today's standards (3).

Early studies demonstrated that although SCCL is a particularly radioresponsive tumor which can be controlled locally by irradiation in a substantial proportion of patients, a total dose similar to that required for the non-small cell subtypes is needed for long-term control. Disease progression in extrathoracic sites remained a major mode of failure, unaffected by local responsiveness to irradiation and the ability of irradiation to control the primary tumor.

Thus, current approaches to treatment of SCCL emphasize systemic chemotherapy to treat the overt or occult distant metastases which exist at presentation. As response rates to various combination chemotherapy programs have increased and median survival times have improved, the necessity of local chest irradiation has been questioned. Numerous studies, both retrospective and prospective, comparing chemotherapy alone to chemotherapy plus irradiation of intrathoracic disease, have yielded conflicting results. The following discussion will evaluate the evidence favoring chest irradiation in a coordinated effort with chemotherapy. It will also address some of the common objections to the use of irradiation of the intrathoracic primary tumor.

What Are the Causes of Death and Failure Patterns in Patients With SCCL Treated With Chest Irradiation Alone or With Inefficacious Chemotherapy?

Causes of death can frequently identify areas in which treatment fails to control disease and may suggest ways to improve therapy. Survival statistics alone may obscure the important clues regarding sites of failure which can be derived from autopsy studies.

The Veterans Administration Lung Group (VALG) conducted randomized trials comparing the effectiveness of thoracic irradiation, with or without lomustine (CCNU) and hydroxyurea, for all histologies of lung cancer. Of 300 autopsies performed on patients treated in the VALG study, 57 showed SCCL. If patients had any evidence of metastasis beyond the thorax, they were designated as dead from "carcinomatosis." Deaths from infection, hemorrhage, or cardiorespiratory failure were felt to represent complications of the primary tumor or involved mediastinal nodes. In 30% of these patients death resulted from uncontrolled intrathoracic tumor without extrathoracic progression (4). An analysis of clinical patterns of failure after radiotherapy alone for patients with SCCL who had LD showed that only 10% died from local recurrence alone, 40% had local plus distant failure, and only 31% died with distant metastases alone (5). However, some of what was termed "local recurrence" may not have been within the field of irradiation. Salazar et al (6,7) reported three of 32 patients (9%) with LD failing within irradiated fields after definitive irradiation alone, with most patients failing at distant sites.

[1]Received Dec 28, 1982; accepted Jan 17, 1983.

[2]Department of Radiation Oncology, The Medical College of Wisconsin Affiliated Hospitals, Milwaukee.

[3]We are grateful to Ms. Barbara Bartos for her assistance in the preparation of this manuscript.

*_Reprint requests to:_ James D. Cox, MD, Department of Radiation Oncology, The Medical College of Wisconsin Affiliated Hospitals, 8700 W Wisconsin Ave, Milwaukee, WI 53226.

What Are the Sites of Failure in Patients With SCCL Treated With Intensive Chemotherapy Alone?

In general, patients treated with intensive chemotherapy alone tend to fail in sites of original disease with or without failure in sites not previously involved, even though the more aggressive chemotherapy has improved response rates. Despite the abundant reports of the results of chemotherapy for SCCL, specific chest failure rates are not always described. Nevertheless, what is available consistently confirms the concept of failure at original sites of involvement. In a recently completed study at the Medical College of Wisconsin (MCW) (8), patients with SCCL received combination chemotherapy with cyclophosphamide (900 mg/m^2), doxorubicin (60 mg/m^2), and vincristine (2 mg/m^2) administered iv every 3 weeks for six cycles. Irradiation of local-regional disease was withheld until: (*a*) failure of continued response during the six cycles, (*b*) any evidence of residual thoracic disease, by chest roentgenography or a computerized tomographic (CT) scan at the end of six cycles, or (*c*) evidence of progression or recurrence at any time. If, after thorough restaging at completion of chemotherapy, the patients were judged to be complete responders, treatment was concluded and the patients were followed. From 1978 to 1979, 31 patients were entered in the study and, of these, 11 of 12 with LD and six of 19 with ED achieved a complete response to the chemotherapy. Of the 17 complete responders, 14 (82%) failed at the primary site from 22 to 87 weeks after the start of therapy. Eight of the 11 complete responders with LD had failure at the primary site, while all six of the complete responders with ED failed at the primary site.

After reviewing the literature available up to 1979, Salazar and Creech (7) noted that the reported incidence of chest failures was 82% in patients with LD treated with chemotherapy alone, if results were combined from the three series in which the chest failure rate was mentioned. In a review of 42 patients with LD treated with chemotherapy alone, Looper et al (9) described failure consistently in the chest. In trials of chemotherapy alone for extensive SCCL, the National Cancer Institute-Veterans Administration reported failure rates of 42%–63% in the chest alone and of 63%–81% in both the chest and distant sites (10,11). These chest relapses occurred despite complete response rates of 55%–69% to the combinations of cyclophosphamide (1000 mg/m^2), CCNU (100 mg/m^2), and methotrexate (15 mg/m^2), with or without other drugs. In a prospective trial of alternating, non-cross-resistance chemotherapy for extensive SCCL, the Mayo Clinic reported total regression rates of 80%–90%, yet 46% of the patients had disease progression first in the original lung tumor. Patients with extensive involvement of liver or lung had disease progression sooner in these sites than those with lesser tumor extent (12).

Harper et al (13) observed a strong correlation between initial intrathoracic tumor size, as assessed by total cross-sectional area on computed tomography, and both response and survival following treatment with combination chemotherapy (cyclophosphamide, methotrexate, and CCNU). When the total tumor area was > 30 cm^2, there were no complete responses, as compared to eight complete responses among 21 patients with a tumor area < 30 cm^2. As tumor size increased, survival was adversely affected. These data suggest that bulky disease sites are not effectively managed with chemotherapy alone and that this failure of disease control decreases survival.

It is frequently argued that the complete response rates are so high with current intensive chemotherapy that irradiation is not needed. Yet, as noted above, about 60%–80% of patients with LD fail in the chest. With complete response rates for LD in the range of 60%–80%, it must be inferred that at least one-half of the chemotherapy failures in LD are due to failure to control the chest primary, with or without concomitant distant dissemination. For ED, complete response rates to intensive chemotherapy fall to 20%–40%. The rate of chest failure without distant failure is 40%–60% of patients with ED, but rises to 60%–80% if those with simultaneous chest disease and distant dissemination are included (7). In the event of a complete response to chemotherapy in a patient with ED, especially with bulky primary tumor, the primary site is frequently the first site of relapse despite continued control of metastatic deposits. This suggests that an increase of overall disease control may result from the addition of a modality of treatment that can increase total cell kill in bulk tumor sites, while relying on chemotherapy for control of occult or minimal to moderate overt disease. Such a modality is logically radiation therapy, since its hallmark is the ability to achieve high rates of local tumor control. Numerous studies of SCCL have related the ability to achieve a complete response to the probability of achieving long-term control; it follows that a true complete response, ie, complete microscopic clearance of tumor, not radiographic clearance, should be the goal of therapy. Radiation therapy can assist in this goal.

Does Radiation Therapy Reduce the Probability of Chest Failure in Patients Receiving Intensive Chemotherapy?

In the review by Salazar and Creech (7) of collected nonrandomized data from the literature, the primary tumor relapse rate was 28% with the addition of radiation therapy to the primary tumor, as compared to an 82% local relapse rate for chemotherapy alone. While

suggestive of a benefit, much of the review data utilized nonintensive chemotherapy and variable radiation therapy.

One of the difficulties in evaluating chest failure rates is the lack of specification of site of chest failure in some reports. Mira and Livingston (14) were among the first to point out that chest relapse does not always mean failure of radiotherapy to control the primary disease. Of 17 patients with LD treated with intensive chemotherapy and irradiation to the primary tumor, only two (12%) showed subsequent disease progression within the radiotherapy portal. However, seven of 17 patients (41%) relapsed in the chest without evidence of regrowth of the primary, suggesting disease extension beyond detectable primary tumor not controlled by chemotherapy. Reseeding of the lung periphery from distant sites of involvement back to the lung may be a factor after systemic relapse. The intrapulmonary spread pattern of SCCL is as yet poorly understood; however, peripheral lung lymphatic involvement (perhaps retrograde) and pleural involvement may be important mechanisms of failure. Lung failure outside radiation therapy fields also reflects understaging and the limits of our existing diagnostic tools, although CT scanning may improve this capability.

As a consequence of the MCW experience noted above with primary site relapse after chemotherapy alone (Study 3), consolidation irradiation was added to the chemotherapy program for complete responders (Study 4). Consolidation consisted of 3750 rads in 15 fractions to the primary tumor volume and regional lymphatics. Although some data suggest that irradiation delivered concurrent with the first cycle(s) of chemotherapy is more effective in terms of cell kill (15), it was elected to sequence the irradiation after complete response to chemotherapy, when tumor cell numbers would theoretically be at a minimum. It was felt that a moderate total dose could then be used to consolidate the chemotherapy program. Previous experience at MCW suggested that moderate radiation doses are effective when combined with intensive chemotherapy (16). In Study 4, patients with a partial response or no response to chemotherapy received therapeutic irradiation of 5000 rads in 5 weeks or palliative irradiation when there was rapid extrathoracic progression.

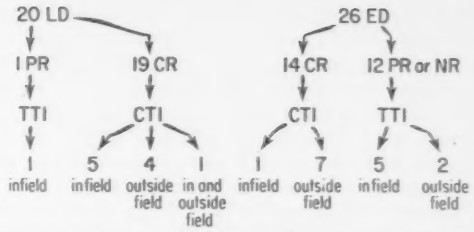


FIGURE 1.—Patterns of chest failure after treatment: CTI for complete responders and therapeutic thoracic irradiation (TTI) for partial responders or nonresponders to combination chemotherapy.

A total of 46 patients from Studies 3 and 4 were eligible for consolidation thoracic irradiation (CTI) as outlined above (fig 1). After thorough initial staging, 20 patients were found to have LD and 26, ED. After intensive chemotherapy, 19 LD and 15 ED patients were found to have a complete response at restaging and all except one ED patient received CTI. With follow-up ranging from 18 to 49 months (median, 32), six of 19 LD patients (31%) and one of 14 ED patients (7%) demonstrated failure within the radiotherapy portal at 24–69 weeks after therapy. Thus, overall, seven of 33

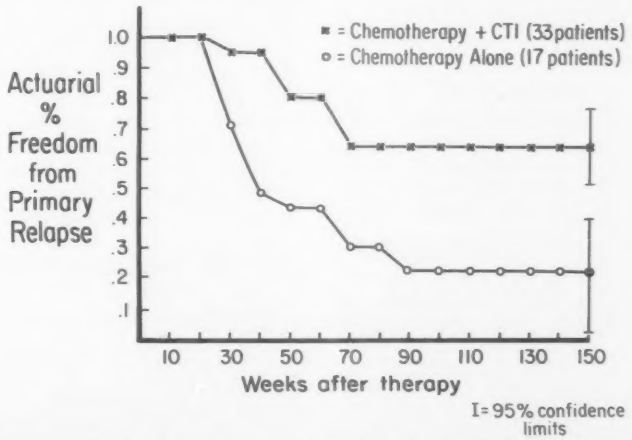


FIGURE 2.—Freedom from primary site relapse with and without CTI in chemotherapy complete responders.

chemotherapy complete responders (21%) who received CTI demonstrated in-field primary tumor relapse.

Figure 2 shows an actuarial comparison of the freedom from primary site relapse between Study 3 without CTI and Study 4 with CTI for patients who were chemotherapy complete responders. There is a significant improvement in the percent of patients continuously free of relapse at the primary site with the addition of CTI.

The chest relapse patterns of all patients receiving chemotherapy and CTI are detailed in figure 1. Of the 31 complete responders, 12 (39%) failed in the chest outside the radiation portal because of pleural disease (in whole or in part) in eight of the 12 instances (75%). Of 12 ED patients who had partial or no response to chemotherapy and who received therapeutic radiation doses to the primary site following chemotherapy, five (42%) failed subsequently within the field and two (17%) failed outside the field. In this group, however, chest relapse was accompanied by nearly simultaneous progression of disseminated disease, and the patients probably did not live long enough to show additional chest failure. These data emphasize the ability of CTI to significantly reduce primary tumor failure but its inability to address disease outside the area treated.

Several other reports of randomized clinical trials show improved local control of primary tumor in LD patients (17–19), as well as ED patients, with the addition of chest irradiation, usually administered as split courses after two to three cycles of chemotherapy. Radiation to the primary, using split courses or other altered fractionation schedules equivalent to < 4000–4500 rads in 4–4$^1/_2$ weeks and given prior to a chemotherapy complete response, is probably inadequate for significant local control. Radiation portals covering the prechemotherapy extent of disease, preferably as outlined by CT scan, are probably essential to prevent local failure.

Does Reduction of Chest Failure Rate by Irradiation Change Survival?

While there is fairly clear evidence that the addition of thoracic irradiation to intensive chemotherapy reduces the rate of primary tumor relapse in SCCL, there is less clear information that this impacts on survival. Several factors hamper analysis of the available data. Much of the thoracic irradiation utilized in conjunction with chemotherapy is compromised in effective biologic dose, in method of administration, or in sequencing with chemotherapy. These alterations are usually made in an effort to reduce the toxicity of the combined program. Furthermore, it is important to evaluate the effect of thoracic irradiation with the correct end point. Median survival determinations may obscure long-term survival, which is an important goal of the treatment program. Nevertheless, there are suggestive bits of information that thoracic irradiation also provides a survival benefit in certain patients. There may be additional bases offered to support the optimism expressed in a recent editorial by Cohen (20) in which he observes that "it might be better to question why combined-modality studies have not yielded better results than to take the pessimistic view that radiation-chemotherapy approaches offer no advantages over chemotherapy alone."

In a preliminary report of a randomized trial, survival was increased after 4000 rads in 15 fractions to the chest primary concurrent with chemotherapy (15). Similarly, the Southwest Oncology Group (SWOG) reported that LD patients receiving chemotherapy plus chest radiotherapy had a longer survival when 4500 rads in 15 fractions, compared to 3000 rads in 10 fractions, was given to the chest. Survival was also correlated to the accuracy of radiotherapy delivery. When irradiation portals were reviewed for accuracy of coverage of tumor-bearing areas and compliance with the protocol-prescribed doses, the group found that LD patients with major violations had a worse survival than those with no violations or minor violations (21). In fact, technical adequacy of the irradiation was the most important prognostic factor.

Choi et al (22) evaluated long-term survival beyond the median in 276 SCCL patients and found that all long-term survivors beyond 24 months had been treated by either chemotherapy plus irradiation to the chest or chest irradiation alone. None of 30 patients treated by chemotherapy alone for LD, with irradiation reserved for local failure, survived > 24 months. In 111 patients with LD at Indiana University, the 4-year absolute survival was 10% for those not receiving primary-directed therapy versus 20% for those receiving primary therapy in addition to chemotherapy ($P = 0.08$) (11).

In MCW Study 4, investigating the influence of CTI, preliminary analysis of actuarial survival after a median follow-up of 18 months was suggestive of a benefit for LD and ED complete responders when compared to Study 3 patients treated with chemotherapy alone (23). The median follow-up of this study is now 31 months for the chemotherapy plus CTI group, and there is a minimum follow-up of 36 months for the chemotherapy-alone group. The absolute survival times are compared in figure 3. At 3 years, four of 34 patients (12%) receiving CTI plus chemotherapy were alive (one with relapse of extrathoracic disease) and one of 17 patients (6%) receiving chemotherapy alone was alive without relapse. This difference is of borderline significance ($P = 0.10$). The median survival of the CTI group is 63 weeks, versus 48 weeks for the chemotherapy alone group ($P = 0.13$, according to Student's t test). While the differences may not reach statistical significance, they may have biologic significance; much larger groups of patients need to be evaluated to achieve conventional statistical significance. To realize the true impact of

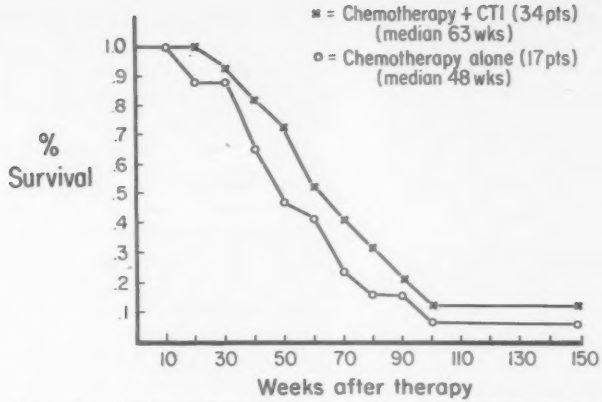


FIGURE 3.—Absolute survival of both LD and ED patients achieving a complete response with and without CTI.

thoracic irradiation on survival it will be necessary to control out-of-field recurrences as well as extrathoracic relapses of bulky metastatic disease. With the added survival imparted by local control of primary disease, the failure patterns may shift (eg, pleural relapses, etc), requiring further change in therapeutic approach.

In the study reported from the National Cancer Institute using chest irradiation simultaneous with remission induction by chemotherapy, significant morbidity and mortality were observed, perhaps related in part to unconventional fractionation of the irradiation. Nevertheless, 25% of LD patients achieved long-term survival (24,25). Greco et al (26) also showed increased survival with combined-modality therapy in LD patients. While improvements in local control and survival of LD patients can be found, there has been little to support improved results in ED patients by the addition of CTI (19,27). However, the fact that a proportion of patients dies with no evidence of tumor beyond the thorax, as determined by autopsy (4), suggests that the effect of thoracic irradiation needs to be evaluated in patients with ED. The intrathoracic tumor is usually the most voluminous, and it is more prone to lethal complications than are metastases to many sites such as bones, subcutaneous tissues, and kidneys.

ARGUMENTS AGAINST THORACIC IRRADIATION IN SCCL

Irradiation May Decrease Bone Marrow Tolerance and Increase Mortality

This is certainly a consideration when the chest is irradiated concurrent with chemotherapy or when irradiation is interspersed with chemotherapy. In this setting, toxicity of treatment may be increased and there is potential interference with marrow tolerance to subsequent cycles of chemotherapy. However, irradiation after complete response to chemotherapy has no effect on marrow tolerance; toxicity has been minimal, and mortality related to toxicity has been nonexistent in the MCW experience.

Several Studies of Chemotherapy Plus Radiotherapy Show No Difference in Chest Failure Rates

As noted previously, many technical problems of radiotherapy administration may account for these results, such as low total doses, split course, sequencing, etc. Furthermore, overall chest recurrences must be separated from true in-field primary tumor relapses. Understaging and incomplete knowledge of local tumor extent may result in incomplete coverage of tumor in the radiation portal. In the MCW experience the addition of thoracic CT scanning identified additional extensions of primary tumor and intrathoracic disease in about 80% of patients (28). The previously mentioned SWOG study corroborates the importance of accuracy of radiotherapy delivery. The excellent local control rate in the MCW series supports the ability of radiation therapy to reduce chest failure rates.

Radiation Therapy Can Be Withheld Until Primary Site Relapse After Chemotherapy With Equivalent Effectiveness

It is axiomatic that irradiation of chemotherapy recurrences is less effective than irradiation of previously untreated tumors. The converse is also true. This principle applies to SCCL treatment. In a review of 23 patients treated in the chest after postchemotherapy progression of the primary tumor, Ihde et al (29) found that only 25% achieved local control by irradiation and that all of them had eventual tumor progression in $2^1/_2$–$3^1/_2$ months. They speculated that radiation doses

higher than those required for consolidation alone were probably necessary in this setting. Whether these higher doses would be as effective and could be delivered with acceptable tolerance is an untested question. The absence of long-term survivors beyond 24 months in Choi's (22) analysis of LD patients treated with chemotherapy alone, with chest irradiation reserved for local failure, reinforces the need to obtain control of the primary tumor during the initial therapy program.

Distant Metastases, Not the Thoracic Tumor, Are the Main Problem

It is perhaps artificial to separate the problems of the intrathoracic tumor and distant metastases, but it is necessary to do so in order to place in perspective the potential role of radiation therapy. Trials investigating the combinations of radiation therapy and chemotherapy have been carried out at the MCW since 1976. A total of 109 consecutive patients with a Karnofsky performance status ≥ 40 have entered these trials. Complete clinical response, defined by repeating all initially abnormal studies after completion of the chemotherapy program (six or eight cycles, depending upon the protocol), has been observed in 57 patients. Thirty-seven patients (19 LD, 18 ED) had tumor control at the primary site and regional lymph nodes; 20 patients (11 LD, nine ED) had progression of the local-regional tumor after complete response. Figure 4 shows the survival experience for the groups with and without progression at the site of the primary tumor. There are too few patients to evaluate LD and ED separately, but these findings suggest that control of the intrathoracic tumor is important for long-term survival, regardless of extent of disease, as long as there is complete response to systemic therapy.

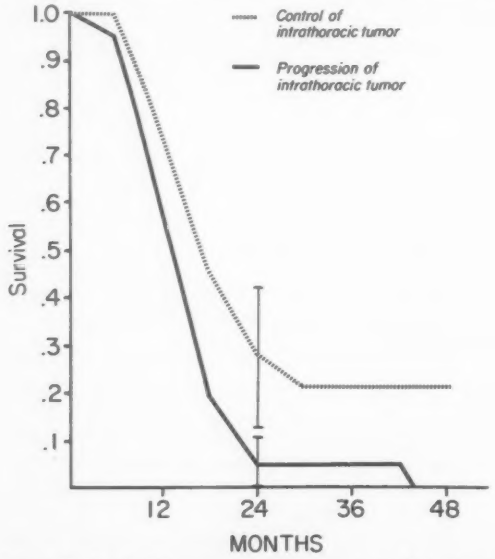


FIGURE 4.—Survival of complete responders by status of intrathoracic tumor.

CONCLUSIONS

Radiation therapy to the chest, adequately encompassing the primary tumor and hilar and mediastinal lymph nodes, significantly improves the control of SCCL within the field of treatment. Although distant metastases are the paramount problem in this disease, the intrathoracic tumor is a major determinant of survival, especially for patients who have a complete response to effective combination chemotherapy. Rather than await major improvements in combination chemotherapy, it is important to design trials to test basic questions of combined-modality therapy; such questions include the optimal dose-time relationships of radiation therapy when combined with effective chemotherapy, the sequencing, and the timing of the two modalities in order to have the greatest antitumor effect and the least morbidity, ie, the best therapeutic ratio.

REFERENCES

1. ROSWIT B, PATNO ME, RAPP R, ET AL. The survival of patients with inoperable lung cancer. A large scale randomized study of radiation therapy vs placebo. AJR 90:688–697, 1968.
2. WOLF J, PATNO ME, ROSWIT B, ET AL. Controlled study of survival of patients with clinically inoperable lung cancer treated with radiation therapy. Am J Med 40:360–367, 1966.
3. FOX W, and SCADDING JG. Medical Research Council comparative trial of surgery and radiotherapy for primary treatment of small cell or oat celled carcinoma of bronchus. Lancet 2:63–65, 1973.
4. COX JD, YESNER R, MIETLOWSKI W, ET AL. Influence of cell type on failure pattern after irradiation for locally advanced carcinoma of the lung. Cancer 44:94–98, 1979.
5. STANLEY K, COX JD, PETROVICH Z, ET AL. Patterns of failure in patients with inoperable carcinoma of the lung. Cancer 47:2725–2729, 1981.
6. SALAZAR OM, RUBIN P, BROWN JC, ET AL. Predictors of radiation response in lung cancer. A clinico-pathological analysis. Cancer 37:2636–2650, 1976.
7. SALAZAR OM, and CREECH RH. "The state of the art" toward defining the role of radiation therapy and the management of small cell bronchogenic carcinoma. Int J Radiat Oncol Biol Phys 6:1103–1117, 1980.
8. BYHARDT RW, LIBNOCH JA, COX JD, ET AL. Local control of intrathoracic disease with chemotherapy and role of prophylactic cranial irradiation in small cell carcinoma of the lung. Cancer 47:2239–2246, 1981.
9. LOOPER JD, JOE BT, SHIDNIA H, ET AL. The role of chest irradiation in limited small cell carcinoma of the lung treated with combination chemotherapy. Int J Radiat Oncol Biol Phys (Suppl 1) 8:69, 1982.
10. COHEN MH, IHDE DC, BUNN PA, ET AL. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 63:163–170, 1979.

11. Cohen MH, Creaven PJ, Fossieck BE, Jr, et al. Intensive chemotherapy with small cell bronchogenic carcinoma. Cancer Treat Rep 61:349–354, 1977.
12. Lininger TR, Fleming TR, and Eagan RT. Evaluation of alternating chemotherapy and sites and extent of disease in extensive small cell lung cancer. Cancer 48:2147–2153, 1981.
13. Harper PG, Souhami RL, Spiro SG, et al. Tumor size, response rate, and prognosis in small cell carcinoma of the bronchus treated by combination chemotherapy. Cancer Treat Rep 66:463–470, 1982.
14. Mira JG, and Livingston RB. Evaluation and radiotherapy implications of chest relapse patterns in small cell lung carcinoma treated with radiotherapy-chemotherapy. Cancer 46:2557–2565, 1980.
15. Cohen MH, Lichter AS, Bunn PA, et al. Chemotherapy-radiotherapy (CT-RT) vs chemotherapy (CT) in limited small cell lung cancer (SCCL) (abstr). Proc Am Assoc Cancer Res and ASCO 21:448, 1980.
16. Cox JD, Byhardt RW, Wilson JF, et al. Dose-time relationships in the local control of small cell carcinoma of the lung. Radiology 128:205–207, 1978.
17. Eagan RT, Maurer LH, Forcier RJ, et al. Combination chemotherapy and radiotherapy in small cell carcinoma of the lung. Cancer 32:371–378, 1973.
18. Fox RN, Woods RL, Brodie GN, et al. A randomized study: small cell anaplastic lung cancer treated by a combination chemotherapy and adjuvant radiotherapy. Int J Radiat Oncol Biol Phys 6:1083–1085, 1980.
19. Williams C, Alexander M, Glatstein EJ, et al. Role of radiation therapy in combination with chemotherapy in extensive oat cell cancer of the lung: a randomized study. Cancer Treat Rep 61:1427–1431, 1977.
20. Cohen MH. Small cell lung cancer: restrained optimism. Int J Radiat Oncol Biol Phys 6:1119–1120, 1980.
21. White JE, Chen T, McCracken J, et al. The influence of radiation therapy quality control on survival, response, and sites of relapse in oat cell carcinoma of the lung—preliminary report of the Southwest Oncology Group study. Proc Am Assoc Cancer Res and ASCO 21:339, 1980.
22. Choi NC, Kaufman SD, Carey RW, et al. Evaluation of the long-term survival of patients with small cell carcinoma of the lung beyond their median survival time in relation to therapeutic modalities and stages of tumor. Int J Radiat Oncol Biol Phys (Suppl 1) 8:70, 1982.
23. Byhardt RW, Cox JD, Holoye PY, et al. The role of consolidation irradiation in combined modality therapy of small cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 8:1271–1276, 1982.
24. Johnson RE, Brereton HD, and Kent CK. Small cell carcinoma of the lung; attempt to remedy causes of the past therapeutic failure. Lancet 1:289–291, 1976.
25. Minna JD, Bunn PA, Carney DN, et al. Experience of the National Cancer Institute (USA) in the treatment and biology of small cell cancer. Bull Cancer (Paris) 69:83–93, 1982.
26. Greco FA, Richardson RL, Snell JD, et al. Small cell lung cancer—complete remission and improved survival. Am J Med 66:625–630, 1979.
27. Dillman RO, Taetle R, Seagren S, et al. Extensive disease small cell carcinoma of the lung. Trial of non-cross resistant chemotherapy in consolidation radiotherapy. Cancer 49:2003–2008, 1982.
28. Norlund J, Byhardt RW, Unger GF, et al. Computed tomography in the diagnosis and staging of small cell lung cancer prior to combined modality therapy. Int J Radiat Oncol Biol Phys (Suppl 1) 8:69, 1982.
29. Ihde DC, Bilek FS, Cohen MH, et al. Response to thoracic radiotherapy in patients with small cell carcinoma of the lung after failure of combination chemotherapy. Radiology 132:443–446, 1979.

Is Thoracic Radiation Therapy Necessary for Patients With Limited-Stage Small Cell Lung Cancer? No.[1]

Martin H. Cohen[2,*]

Review of the literature on treatment of small cell cancer of the lung (SCCL) allows the following conclusions to be drawn. Radiation therapy is an active treatment modality in SCCL (1,2). Multiple chemotherapy agents are also active in this disease (3–5). Radiation therapy can cure a small percentage of patients who clinically have disease confined to one hemithorax (6–9). Combination chemotherapy can achieve similar therapeutic results (10,11). Radiation therapy alone or combined with chemotherapy may produce long-duration local control of "bulk" intrathoracic disease (12,13). Combination chemotherapy alone is less effective in controlling intrathoracic disease (12,13). Combined-modality (chemotherapy plus radiation therapy) induction treatment may be associated with significant acute local and systemic treatment toxicity and with treatment-related mortality (14,15). Chemotherapy generally produces less severe acute toxicity (16,17). Combined-modality treatment may result in debilitating chronic toxicity (18,19). Chronic chemotherapy toxicity is qualitatively and quantitatively less severe (18).

From the above information it might intuitively be concluded that combined-modality treatment would produce superior survival results in limited-stage SCCL patients (because of increased local control) at a cost of increased treatment-related morbidity and mortality. Unfortunately, these assumptions are not conclusively borne out by clinical trial results.

In critically evaluating survival curves of SCCL therapy trials the growth kinetics of this neoplasm must be kept in mind. While early reports, based on small numbers of patients, indicated a mean tumor doubling time of about 1 month (20), more recent studies, with larger numbers of patients, indicate that the clinical doubling time is approximately 2–3 months (21). Implications of doubling times in this range, with regard to the duration of complete remission, have been discussed by Shackney et al (21). A patient whose tumor burden is reduced by therapy to 1×10^6 cells could remain in complete remission for ≥ 2 years before relapse. Patients with lesser tumor burdens might remain in complete remission for ≥ 5 years before relapse. Clinical verification of these kinetic predictions comes from several sources, including a registry of 97 long-term (> 2.5 years) surviving SCCL patients (6). Twenty-one of these patients developed recurrent disease. Eight patients died of progressive disease 30–33 months after diagnosis, while recurrent disease was noted in another 11 patients 34–51 months after diagnosis. Two patients treated with surgery alone died of recurrent disease 8 and 9 years, respectively, after their initial surgery. Similar observations are made in clinical trials with sufficiently long patient follow-up. Patient deaths are continually observed during the first 3 years of follow-up; if survival curves ever plateau, they do so only beyond that time (19,22,23).

Based on the above considerations most literature reports are uninterpretable with regard to the percentage of patients achieving long-duration survival, because of early data reporting. Three examples, out of innumerable possible choices, of the false optimism generated by early data reporting will be cited. Johnson et al reported the results of combined-modality therapy of limited-stage SCCL on three occasions. In 1976 (24), with 11 patients in the study and with a median patient follow-up of about 1 year, the actuarial survival curve predicted a 90% long-duration survival. In 1978 (14), with 36 patients in the study and with a similar median patient follow-up, the survival curve plateaued at 40% long-duration survival. In 1981 (23), with 42 limited-stage disease patients in the study and a median follow-up of 36 months, the actuarial curve plateaued at 10% long-duration survival (the actual survival was 19%).

Greco et al used a remission induction combined-mo-

[1]Received Sept 15, 1982; accepted Jan 18, 1983.

[2]Oncology Section, Veterans Administration Medical Center, Washington, DC.

*Reprint requests to: Martin H. Cohen, MD, Oncology Section, Veterans Administration Medical Center, 50 Irving St, NW, Washington, DC 20422.

dality regimen that was similar to, although slightly less aggressive than, that used by Johnson. In 1978 (25), with 16 patients in the study and a median patient follow-up of 14 months, the actuarial survival curve plateau indicated 80% long-duration survivors. In 1979 (26), with 32 patients in the study and with a similar median follow-up time, the survival curve plateaued at 53% long-duration survivors. In 1980 (27), with 31 patients in the study and a median follow-up of 24 months, the plateau was at 20% long-duration survival.

Studies employing chemotherapy alone in limited small cell carcinoma can also be faulted for early data reporting. In a trial, first published in abstract form (28), that included 61 SCCL patients treated with cyclic alternating therapy with two or three non-cross-resistant drug combinations, it was reported that the potential 2-year disease-free survival for complete responders was 29%. When the manuscript was published, after a 28-month median patient follow-up, the actuarial survival plot plateaued at 23% long-duration survival for complete responders (29). Currently, only two (7%) of the complete responders remain alive at 79 and 84 months from start of therapy; a third patient relapsed and died 79 months from the start of therapy.

Accepting that early data reporting prevents accurate determination of long-duration survival, is there any evidence for survival benefit of combined-modality treatment versus chemotherapy alone in limited-disease patients? Several reviews, summarizing pertinent treatment literature, have recently been published (15,30–34). These reviews highlight the paucity of randomized trials addressing this question. Only three such trials have been published, and those appear in abstract form (35–37). In none of these trials was one treatment arm significantly better than the other. In evaluating nonrandomized trials of chemotherapy alone or of combined-modality treatment, median survival and long-duration survival are more objective parameters than are complete and partial response rates. In limited-disease patients median survival is generally in the range of 12–14 months, irrespective of therapy. Projected long-duration survival is in the range of 10%–20%, with combined-modality studies often predicting survival rates closer to 20% and studies of chemotherapy alone predicting closer to 10%. Difficulty in determining the accuracy of these predictions has already been discussed. Further, there may be considerable differences in patient population in different studies. There is no single, standard definition of limited disease. Patients with bilateral mediastinal, supraclavicular, and bronchoscopic disease and with an ipsilateral pleural effusion are considered to have limited disease in some studies but not in others. Patient prognostic factors may also vary from one study to another. Besides performance status, other important prognostic factors include cigarette smoking status, tumor size, patient sex, and certain pretreatment laboratory parameters. Johnston-Early et al (38) separated SCCL patients into three categories based on their cigarette smoking behavior: patients who permanently stopped smoking prior to diagnosis, patients who permanently stopped smoking at diagnosis, and those who continued to smoke. Patients received equivalent therapy and were comparable with regard to performance status, disease stage, and immune reactivity. Long-duration survival of chemotherapy-treated patients was noted only in those individuals who had stopped smoking. While tumor size is often difficult to determine accurately on chest roentgenogram (39), it appears that large tumors have a worse response than small tumors to either chemotherapy or radiation therapy (40,41). The sex ratio of patients included in a study may also be important, since females have a somewhat better prognosis than males (22,42). Pretreatment laboratory parameters have been shown by two groups to provide independent prognostic information (43,44); patients with high plasma hemoglobin, high serum albumin, and low serum lactate dehydrogenase levels survive significantly longer than do patients with the opposite results.

In the introduction it was stated that combined-modality therapy was intuitively expected to prolong survival compared to chemotherapy alone, because of improved local control. Why has this not been borne out? Perhaps both chemotherapy and radiation therapy kill primarily the same tumor cell population, ie, those cancer cells located in the well-vascularized, peripheral portion of the tumor mass. Tumor cells in the more central regions of the tumor may be resistant to moderate-dose radiation therapy because of hypoxia and may be resistant to chemotherapy because of poor drug delivery to this area (45,46). Long-duration local control of SCCL with megavoltage radiation therapy alone appears to require radiation doses of $\geq$ 5000 rad. The two megavoltage-treated 10-year survivors in the British Medical Research Council trial of radiation therapy versus surgery received 4970 rad in 30 days and 5200 rad in 28 days, respectively (7). Similarly, the Veterans Administration Lung Group treated patients with limited-stage SCCL with 5000–6000 rad in 5–6 weeks. Four of 33 patients survived 24 months (8). Lee et al (9), treating 58 SCCL patients with continuous or split-course radiation therapy in equivalent dose, had a similar percentage of 5-year survivors. Rissanen et al (47), in an autopsy study, found that six of 17 patients who received radiation doses of 4800–6250 rad had no evident residual disease. Choi and Carey (48) demonstrated a dose-response relationship for local control by radiation therapy. Unfortunately, the follow-up period was only 4 months. Treatment with 3000 rad (1133 rets) yielded local control in 60%, as compared to 79% local control with 4000 rad (1361 rets) and 88% local control with 4800 rad (1518 rets). Cox and colleagues (49,50) similarly demonstrated that when radiation therapy was

used alone, approximately 1700 rets was required for local control. With combined-modality treatment, however, local control was often achieved with 1300 rets. Median time of patient follow-up was not mentioned in this study but many patients were followed for < 26 weeks (49). The findings of Cox et al had important influence on the design of combined-modality treatment programs. In numerous studies the radiation dose to the primary tumor and to the mediastinum was in the range of 3000–4000 rad. In the few studies employing higher radiation doses, treatment compromises were made, including delay of chemotherapy administration for long periods of time (41,51) or a long interval between courses of radiation therapy (52,53). Other compromises are routinely made when radiation therapy is combined with chemotherapy. Initial field size may be limited to residual tumor volume after one or more courses of chemotherapy to "spare" as much lung as possible. Shrinking-field technique may be used for the same purpose. Posterior blocks may be used to limit radiation dose to the esophagus and spinal cord. Whether any or all of the compromises account for failure of combined-modality treatment to significantly improve survival in SCCL remains to be determined.

Despite compromises made in radiation therapy doses, and often also in chemotherapy doses during radiation therapy, significant acute toxic effects are seen with combined-modality treatment that are quantitatively greater than with chemotherapy alone or are unique to combined-modality treatment. In a healthy adult, ribs, sternum, and scapula comprise 15%–20% of functioning bone marrow (54). Although the radiation therapy dose required to make bone marrow permanently aplastic is not known, delayed regeneration of sternal marrow was noted after 3000 rad in 3 weeks (55). Abrams and coworkers (56) studied 15 patients with limited-stage SCCL who were randomized to either chemotherapy alone or chemotherapy with concomitant radiation therapy. They found that the latter group had significantly lower wbc count nadirs than did the former. In addition, recovery of peripheral blood colony-forming units after chemotherapy was markedly suppressed in patients receiving radiation therapy. Destruction of hematopoietic stem cells both within the marrow encompassed by the radiation port and within the peripheral blood circulating through the treatment port was postulated.

Other significant acute toxic effects generally associated with combined-modality treatment include desquamative skin reactions, radiation esophagitis, and diffuse interstitial pulmonary disease. These toxic effects have recently been reviewed by Aisner and Wiernik (18).

In addition to acute toxic effects, combined modality-treated patients are at risk for developing serious chronic toxic effects, including radiation myelitis (1), esophageal stricture (24,57), leukemia (58), and chronically worsening pulmonary function tests (59–61). The latter is evidenced by decreased lung compliance, decrease in static lung volumes, decreased diffusing capacity, increased alveolar-arterial oxygen gradient, and progressively increasing arterial hypoxia (59). Nearly all of these changes result from combined modality-induced pulmonary fibrosis with thickening of alveolar walls, decrease in pulmonary capillaries, and gross destruction of alveolar architecture. The physiologic consequences of these abnormalities have been addressed by Ellison et al (19), who reported on ten patients treated with combined-modality therapy who were alive and disease-free 35–66 months after start of therapy. Only two patients were able to return to work. Most of the other patients were relatively disabled because of exertional dyspnea or because of unusual neurologic sequelae presumed related to therapy.

In summary, currently utilized combined-modality therapy approaches in limited-stage SCCL produce identical median survival to that achieved with chemotherapy alone. It is possible, though not conclusively proven, that combined-modality therapy may slightly increase the possibility of long-duration survival. The price paid for this possible survival improvement is increased treatment-related morbidity and mortality. Is the price worth it? McNeill and co-workers (62,63) have developed a model system to evaluate such questions. They show that even if combined-modality therapy were better than chemotherapy alone, many patients, if given an informed choice, would be averse to receiving it because of increased acute morbidity and mortality and because of increased chronic morbidity relative to chemotherapy alone.

Finally, it is necessary to comment on the efficacy of combination chemotherapy in SCCL. At present, numerous combinations of clinically active agents have been studied. Intensity of drug treatment has varied, with some studies using drug doses and schedules suitable for outpatient treatment and others using induction regimens comparable in intensity to treatments used in acute nonlymphocytic leukemia. While these treatments have definitely increased median survival, relapses, even in complete responders, generally occur within 1 year. The conclusion must be that present-day chemotherapy, in most patients, decreases the pretreatment tumor cell population by only a few logs. It seems unlikely that much future improvement will result from continued empiric attempts at combining "active" drugs. New drugs, or innovative ways of using available drugs, are desperately needed.

REFERENCES

1. Seydel HG, Creech RH, Mietlowski W, et al. Radiation therapy in small cell lung cancer. Semin Oncol 5:288–298, 1978.
2. Salazar OM, and Creech RH. "The state of the art" toward

defining the role of radiation therapy in the management of small cell bronchogenic carcinoma. Int J Radiat Oncol Biol Phys 6:1103–1117, 1980.
3. Broder LE, Cohen MH, and Selaway OS. Treatment of bronchogenic carcinoma. II. Small cell cancer. Cancer Treat Rev 4:219–260, 1977.
4. Weiss RB. Small cell carcinoma of the lung: therapeutic management. Ann Intern Med 88:522–531, 1978.
5. Sierocki JS, Hilaris BS, Hoffan S, et al. *cis*-Dichlorodiammineplatinum(II) and VP-16-213: an active induction regimen for small cell carcinoma of the lung. Cancer Treat Rep 63:1593–1597, 1979.
6. Matthews MJ, Rosencweig M, Staquet MH, et al. Long term survivors with small cell carcinoma of the lung. Eur J Cancer 16:527–531, 1980.
7. Fox W, and Scadding JG. Medical Research Council comparative trial of surgery and radiotherapy for primary treatment of small-celled, or oat-celled carcinoma of bronchus: ten-year follow-up. Lancet 2:63–65, 1973.
8. Petrovich Z, Mietlowski W, Ohanian M, et al. Clinical research on the treatment of locally advanced lung cancer. Cancer 42:1129–1134, 1978.
9. Lee RE, Carr DT, and Childs DS, Jr. Comparison of split course radiation therapy and continuous radiation therapy for unresectable bronchogenic carcinoma: 5-year results. AJR 126:116–122, 1976.
10. Cohen MH. Small cell bronchogenic carcinoma—a prolonged remission following chemotherapy. JAMA 237:2529, 1977.
11. Hansen M, Hansen HH, and Dombernowsky P. Long term survival in small cell carcinoma of the lung. JAMA 244:247–250, 1980.
12. Ajaikumar BS, and Barkley HT, Jr. The role of radiation therapy in the treatment of small cell undifferentiated bronchogenic carcinoma Int J Radiat Oncol Biol Phys 5:977–982, 1979.
13. Matthews MJ. Effects of therapy on the morphology and behavior of small cell carcinoma of the lung—a clinicopathologic study. *In* Lung Cancer: Progress in Therapeutic Research (Muggia F, and Rozencweig M, eds). New York, Raven Press, 1979, pp 155–165.
14. Johnson RE, Brereton HD, and Kent CH. "Total" therapy for small cell carcinoma of the lung. Ann Thorac Surg 25:510–515, 1978.
15. Bunn PA, Jr, and Ihde DC. Small cell bronchogenic carcinoma: a review of therapeutic results. *In* Lung Cancer (Livingston RB, ed). Boston, Martinus Nijhoff, 1981, pp 169–208.
16. Abeloff MD, Ettinger DS, Order SE, et al. Intensive induction chemotherapy in 54 patients with small cell carcinoma of the lung. Cancer Treat Rep 65:639–646, 1981.
17. Valdivieso M, Tenczynski TF, Rodriguez V, et al. Chemoimmunotherapy of small cell bronchogenic carcinoma with VP 16-213, ifosfamide, vincristine, adriamycin and *Corynebacterium parvum*. Cancer 48:238–244, 1981.
18. Aisner J, and Wiernik PH. Complications of treatment and of improved survival in patients with small cell carcinoma of the lung. *In* Small Cell Lung Cancer (Greco FA, Oldham RK, and Bunn PA, Jr, eds). New York, Grune and Stratton, 1981, pp 381–398.
19. Ellison N, Bernath A, Kane R, et al. Disturbing problems of success: clinical status of long-term survivors of small cell lung cancer. Proc ASCO 1:149, 1982.
20. Straus MJ. The growth characteristics of lung cancer and its application to treatment design. Semin Oncol 1:167–174, 1974.
21. Shackney SE, Straus MJ, and Bunn PA, Jr. *In* Small Cell Lung Cancer (Greco FA, Oldham RK, and Bunn PA, Jr, eds). New York, Grune and Stratton, 1981, pp 225–234.
22. Maurer LH, and Pajak TF. Prognostic factors in small cell carcinoma of the lung: a Cancer and Leukemia Group B study. Cancer Treat Rep 65:767–774, 1981.
23. Catane R, Lichter A, Lee YJ, et al. Small cell lung cancer: analysis of treatment factors contributing to prolonged survival. Cancer 48:1936–1943, 1981.
24. Johnson RE, Brereton HD, and Kent CH. Small cell carcinoma of the lung: attempt to remedy causes of past therapeutic failure. Lancet 2:289–291, 1976.
25. Greco FA, Richardson RL, and Schulman SF. Treatment of oat cell carcinoma of the lung: complete remission, acceptable complications, and improved survival. Br Med J 2:10–11, 1978.
26. Greco FA, Richardson RL, Snell JD, et al. Small cell lung cancer. Complete remission and improved survival. Am J Med 66:625–630, 1979.
27. Oldham RK, and Greco FA. Small cell lung cancer. A curable disease. Cancer Chemother Pharmacol 4:173–177, 1980.
28. Cohen MH, Ihde DC, Fossieck BE, et al. Intensive chemotherapy of small cell bronchogenic carcinoma. Proc Am Assoc Cancer Res and ASCO 18:286, 1977.
29. Cohen MH, Ihde DC, Bunn PA, Jr, et al. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 63:163–170, 1979.
30. Livingston RB. Small cell carcinoma of the lung. Blood 56:575–584, 1980.
31. Weiss RB, Minna JD, Glatstein E, et al. Treatment of small cell undifferentiated carcinoma of the lung: update of recent results. Cancer Treat Rep 64:539–548, 1980.
32. Greco FA, Einhorn LH, Richardson RL, et al. Small cell lung cancer: progress and perspectives. Semin Oncol 5:323–335, 1978.
33. Bunn PA, Jr, Lichter AS, Glatstein E, et al. Results of recent studies in small cell bronchogenic carcinoma and prospects for future studies. *In* Small Cell Lung Cancer (Greco FA, Oldham RK, and Bunn PA, Jr, eds). New York, Grune and Stratton, 1981, pp 413–446.
34. Ihde DC, and Bunn PA, Jr. Chemotherapy of small cell bronchogenic carcinoma. *In* Recent Advances in Clinical Oncology (Williams CJ, and Whitehouse JMA, eds). New York, Churchill Livingstone, 1982, pp 305–323.
35. Hansen HH, Dombernowsky P, Hansen HS, et al. Chemotherapy versus chemotherapy plus radiotherapy in regional small cell carcinoma of the lung. A randomized trial. Proc Am Assoc Cancer Res and ASCO 20:277, 1979.
36. Stevens E, Einhorn L, and Rohn R. Treatment of limited small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 20:435, 1979.
37. Cohen MH, Lichter AS, Bunn PA, Jr, et al. Chemotherapy-radiation therapy versus chemotherapy in limited small cell cancer. Proc Am Assoc Cancer Res and ASCO 21:464, 1980.
38. Johnston-Early A, Cohen MH, Minna JD, et al. Smoking abstinence and small cell lung cancer survival. An association. JAMA 244:2175–2179, 1980.
39. Eagan RT, Fleming TR, and Schoonover V. Evaluation of response criteria in advanced lung cancer. Cancer 44:1125–1128, 1979.
40. Harper PG, Souhami RL, Spiro SG, et al. Tumor size, response rate, and prognosis in small cell carcinoma of the bronchus treated by combination chemotherapy. Cancer Treat Rep 66:463–470, 1982.
41. Mira JG, and Livingston RB. Evaluation and radiotherapy implications of chest relapse patterns in small cell lung carcinoma treated with radiotherapy-chemotherapy. Cancer 46:2557–2565, 1980.
42. Ettinger DS, and Lagakos S. Phase III study of CCNU, cyclophosphamide, adriamycin, vincristine, and VP-16 in small cell carcinoma of the lung. Cancer 49:1544–1554, 1982.
43. Cohen MH, Makuch RW, Johnston-Early A, et al. Laboratory parameters as an alternative to performance status in prognostic stratification of patients with small cell lung cancer. Cancer Treat Rep 65:187–195, 1981.
44. Koretz MM, and Carter SK. Laboratory parameters as alternative to performance status in predicting survival in oat cell carcinoma. Proc ASCO 1:154, 1982.
45. Tannock IF. The relation between cell proliferation and the vascular system in a transplanted mouse mammary carcinoma. Br J Cancer 22:258–273, 1968.
46. Gullino PM. The internal milieu of tumors. Prog Exp Tumor Res 8:1–25, 1966.
47. Rissanen PM, Tikka U, and Holsti LR. Autopsy findings in lung cancer treated with megavoltage radiotherapy. Acta Radiol Ther Phys Biol 7:433–442, 1968.

48. Choi CH, and Carey RW. Small cell anaplastic carcinoma of lung. Reappraisal of current management. Cancer 37:2651–2657, 1976.
49. Cox JD, Byhardt RW, Wilson JF, et al. Dose-time relationships and the local control of small cell carcinoma of the lung. Radiology 128:205–207, 1978.
50. Cox JD, Byhardt RW, Komaki R, et al. Interaction of thoracic irradiation and chemotherapy on local control and survival in small cell carcinoma of the lung. Cancer Treat Rep 63:1251–1255, 1979.
51. Holoye PY, and Samuels ML. Cyclophosphamide, vincristine and sequential split-course radiotherapy in the treatment of small cell lung cancer. Chest 67:675–679, 1975.
52. McMahon LJ, Herman TS, Manning MR, et al. Patterns of relapse in patients with small cell carcinoma of the lung treated with adriamycin-cyclophosphamide chemotherapy and radiation therapy. Cancer Treat Rep 63:359–362, 1979.
53. Livingston RB, Moore TN, Heilbrun L, et al. Small cell carcinoma of the lung. Combined chemotherapy and radiation. Ann Intern Med 88:194–199, 1978.
54. Rubin PH, and Scarantino CH. The bone marrow organ: the critical structure in radiation/drug interaction. Int J Radiat Oncol Biol Phys 4:3–23, 1978.
55. Sykes MP, Chu FCH, Savel H, et al. The effects of various dosages of irradiation upon sternal marrow regeneration. Radiology 83:1084–1087, 1964.
56. Abrams RA, Lichter A, Johnston-Early A, et al. Enhanced suppression of peripheral blood CFUc and white blood cell platelet counts by regional radiotherapy in adult patients with non-hematologic malignancy. Proc Am Assoc Cancer Res and ASCO 20:144, 1979.
57. Chabora BM, Hoffan S, and Wittes R. Esophageal complications in the treatment of oat cell carcinoma with combined irradiation and chemotherapy. Radiology 123:185–187, 1977.
58. Bradley EC, Schechter GP, Matthews MJ, et al. Erythroleukemia and other hematologic complications of intensive therapy in long-term survivors of small cell lung cancer. Cancer 49:221–223, 1982.
59. Gross NJ. Pulmonary effects of radiation therapy. Ann Intern Med 86:81–92, 1977.
60. Einhorn L, Krause M, Hornback N, et al. Enhanced pulmonary toxicity with bleomycin and radiotherapy in oat cell lung cancer. Cancer 37:2414–2416, 1976.
61. Lamoureux KB. Increased clinically symptomatic pulmonary radiation reactions with adjuvant chemotherapy. Cancer Chemother Rep 58:705–708, 1974.
62. McNeill BJ, Weichselbaum R, and Pauker SG. Fallacy of five-year survival in lung cancer. N Engl J Med 299:1397–1401, 1978.
63. ———. Speech and survival. Tradeoffs between quality and quantity of life in laryngeal cancer. N Engl J Med 305:982–987, 1981.

Strategies for the Prevention of Infection in the Myelosuppressed or Immunosuppressed Cancer Patient[1]

Philip A. Pizzo[2,*] and Stephen C. Schimpff[3]

An approach to infection prevention depends upon an understanding or at least a recognition of those factors which tend to predispose the myelosuppressed or immunosuppressed cancer patient to infection. Among the most common predisposing factors are: granulocytopenia, usually in association with some degree of damage to an anatomic barrier to microorganisms; cellular immune dysfunction; humoral immune dysfunction; obstruction to natural passages; central nervous system dysfunction; or the iatrogenic procedures common to medical practice (table 1). The patterns of infection tend to vary in each of these settings. For example, the patient with profound granulocytopenia most frequently develops infections along the alimentary canal, especially at areas of mucosal damage; the respiratory tract, perhaps because of damage to the ciliary function; and areas where the integument has been damaged, including intravenous sites or sites of invasive procedures. The organisms which cause infection in such patients are usually those which have colonized at or in proximity to these sites and are generally the aerobic gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* or the gram-positive cocci such as *Staphylococcus aureus*. In addition, patients who have received broad-spectrum antibiotics for prolonged periods are at increased risk for infections with fungi such as *Candida albicans* and *Aspergillus fumigatus*.

On the other hand, the patient whose primary abnormality is a deficiency of cellular immunity tends to develop infections with a distinctly different group of organisms, notably those thought of as the obligate intracellular pathogens. These include: the bacteria *Listeria monocytogenes*, *Salmonella* species, *Mycobacterium* species, *Nocardia asteroides*, and *Legionella pneumophilia;* the fungi *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Coccidioides immitis;* the viruses varicella-zoster, herpes simplex, and cytomegalovirus (CMV); the protozoa *Pneumocystis carinii* and *Toxoplasma gondii;* and the helminth

TABLE 1.—Factors predisposing to infection among patients with cancer

Granulocytopenia (eg, acute leukemia)
Usually with associated damage to body barriers (especially alimentary canal mucosa, respiratory tract ciliary function, and integument)
Common organisms
Gram-negative bacilli: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*
Gram-positive cocci: *Staphylococcus aureus* and *Staphylococcus epidermidis*
Yeasts: *Candida* spp., *Torulopsis glabrata*
Fungi: *Aspergillus* spp., *Mucor*
Cellular immune deficiency (eg, lymphoma)
Common organisms
Bacteria: *Listeria monocytogenes*, *Salmonella* spp., *Mycobacterium* spp., *Nocardia asteroides*, and *Legionella pneumophilia*
Viruses: varicella-zoster, herpes simplex, and cytomegalovirus (CMV)
Fungi: *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Coccidioides immitis*
Protozoa: *Pneumocystis carinii* and *Toxoplasma gondii*
Helminth: *Strongyloides stercoralis*
Humoral immune dysfunction (eg, multiple myeloma)
Common organisms: *Streptococcus pneumoniae* and *Hemophilus influenzae*
Obstruction to natural passages (eg, solid tumors)
Common sites: respiratory tract, biliary tract, and urinary tract
Common organisms: locally colonizing
Central nervous system dysfunction (eg, brain tumors)
Common sites: pneumonitis and urinary tract infection
Common organisms: locally colonizing
Infections associated with medical procedures
Procedures: intravascular catheters, urinary catheters, and respiratory assist devices
Common organisms: locally colonizing

[1]Received Dec 28, 1982; accepted Jan 6, 1983.

[2]Infectious Disease Section, Pediatric Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[3]Section of Infection and Microbiological Research, University of Maryland Cancer Center and the Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, Baltimore.

*Reprint requests to: Philip A. Pizzo, MD, Pediatric Branch, National Cancer Institute, Bldg 10, Rm 13C101, Bethesda, MD 20205.

Strongyloides stercoralis. These infections, although uncommon, tend to occur in patients with diseases such as Hodgkin's disease or in patients who have received a bone marrow transplant.

A distinctly different pattern of infection occurs among patients whose primary abnormality is a deficiency in humoral immune function. For example, the patient with multiple myeloma who has decreased opsonizing antibody is at increased risk for infection with organisms like *Streptococcus pneumoniae* and *Hemophilus influenzae*.

As with any pathologic situation, partial obstruction to a natural passage can lead to a postobstructive infection, such as the pneumonitis seen with obstruction of a bronchus, the urinary infection with an obstruction of the urinary tract, etc. Central nervous system lesions, be they organic or metabolic, lead to abnormalities of the gag reflex with the potential for aspiration pneumonitis, while abnormalities of micturition may result in the development of urinary tract infections. Naturally, there is always the risk of infection associated with intravascular catheters, respiratory assist devices, or urinary catheters.

Hence, to establish an effective program for the prevention of infectious complications in the immunosuppressed cancer patient, the patient's underlying disease, the treatment status, and a large array of potential pathogens must be considered. As shown in table 2, the major pathogens against which prevention strategies have been targeted include gram-negative and gram-positive bacteria, fungi, parasites, and viruses. Prevention strategies must also take into account the manner by which these organisms are acquired and lead to infection in the cancer patient. As was shown by Schimpff and colleagues (1) at the University of Maryland Cancer Center, colonization is an important prerequisite to infection, since > 80% of the organisms causing infection in the cancer patient can be found in the host's endogenous microbial flora, although nearly 50% of these organisms are acquired while the patient is in the hospital (fig 1). Colonization is promoted by the altered epithelial binding of organisms to the oropharynx and other mucosal surfaces as a consequence of the patient's underlying disease and is enhanced by perturbations of the microenvironment due to antimicrobial and chemotherapeutic agents (2–4). The sources for colonization

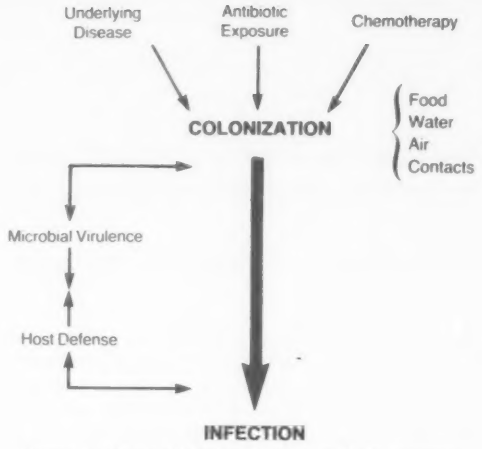


FIGURE 1.—Origin of infection in cancer patients: exposure-colonization-infection.

of the hospitalized patient are ubiquitous and include food (as a source of gram-negative bacteria from fresh fruits and vegetables or dairy products) (5,6); water (as a source of gram-negative rods from faucet aerators or ice machines and more recently as a source of *Legionella* from water-cooling tanks or shower heads); air (which carries bacterial and viral droplets as well as the spores of *Aspergillus* found in construction and fireproofing material) (7,8); and perhaps most importantly, transmission of potential pathogens from patient to patient by medical personnel who all too frequently neglect simple handwashing (9–11). Once a new microorganism has been acquired, the risk for developing an infection results from the balance between the inherent virulence properties of the organism and the degree of host compromise. For example, colonization with *Pseudomonas aeruginosa* or *Candida tropicalis* is more likely to result in infection than is colonization with less virulent organisms such as *Escherichia coli* or even *Candida albicans* (12,13). Nonetheless, if the degree of host impairment is sufficiently profound, virtually any organism can become a serious pathogen. In addition, latent organisms, such as *Pneumocystis carinii* or herpesviruses, must also be considered, since these organisms can be reactivated during immunosuppressive therapy

TABLE 2.—Major microorganisms against which prophylaxis should be aimed

Bacteria	Fungi	Protozoa	Helminths	Viruses
Enterobacteriaceae	*Candida* spp.	*Pneumocystis carinii*	*Strongyloides stercoralis*	Herpes simplex
Pseudomonads	*Aspergillus* spp.			Varicella-zoster
Staphylococci	*Torulopsis glabrata*			CMV
Streptococci				

and either cause infection themselves or further suppress immune defenses resulting in secondary bacterial infections (as has been shown for CMV in patients undergoing cardiac transplantation) (14,15). Hence, in defining a program for infection prevention, one must consider both exogenous and endogenous microorganisms as well as the patient's underlying disease and the type of therapy which is being administered. For example, the cancer patient undergoing induction chemotherapy is primarily at risk for bacterial infections, although, if the period of granulocytopenia is protracted and is associated with prolonged antibiotic administration, the risk for developing a fungal infection increases. Patients receiving maintenance therapy are also at risk for bacterial infections if they become granulocytopenic, but infection with *Pneumocystis carinii* or viruses may become more important considerations if the maintenance therapy continues for months or years. Similarly, patients with lymphoma treated with irradiation have a high risk of herpes zoster developing during the ensuing year of immunosuppression (16). In contrast, patients undergoing allogenic bone marrow transplantation are at risk for infection with virtually all classes of microorganisms, although bacteria and fungi predominate during the preparative and immediate posttransplant period, *Pneumocystis carinii* and viral infections (especially CMV) are most frequent during the initial postengraftment period, and *Streptococcus pneumoniae* infection (17) becomes more common a year or more following transplantation as prior antibody is depleted and not renewed. Thus, the transplant patient passes through successive stages of infection risk due initially to granulocytopenia and associated mucosal damage, then to cellular immune deficiency, and then to overlapping humoral immune deficiency.

Given this background on the predisposing factors to infection and the organisms and sites at which infection is likely to occur, one can approach the development of prevention strategies by considering four separate but interrelated techniques (18). These include (*a*) bolstering the host defense mechanisms, (*b*) reducing damage to natural body barriers, (*c*) reducing the acquisition of new organisms from the environment, and (*d*) suppressing those potentially pathogenic organisms currently colonizing the patient (table 3). A logical approach to infection prevention must include all four of these approaches for an effective overall program. Unfortunately, many of the simple techniques which can be utilized in any hospital setting have often been overlooked and much greater emphasis has been placed on approaches which are complex or difficult for the patient and staff to comply with. Similarly, emphasis is often placed on a single modality such as antimicrobial flora suppression, probably because such an approach can be "ordered" by the physician, whereas other approaches of equal or greater importance, such as handwashing, are often ignored or omitted from an infection prevention program.

TABLE 3.—Approaches to infection prevention

Bolster host defense mechanisms
Achievement of complete remission as rapidly as possible
Vaccines (influenza, pneumococcal, *Pseudomonas*, common antigen against "J5")
Leukocyte transfusions
Immunomodulators (eg, lithium carbonate)
Nutrition
Avoid damage to body barriers
Care with venipuncture, bone marrow aspiration
Avoid invasive procedures (iv or urinary catheters)
Reduce acquisition of new potential pathogens
Attention to careful handwashing
Low microbial food and beverages
Control of water supplies, ice machine
Attention to proper housekeeping procedures
Laminar air flow room isolation
Suppress colonizing organisms
Total microbial suppression (oral nonabsorbable antibiotics + skin antisepsis with or without systemic antibiotics)
Selective microbial suppression (preferably while maintaining colonization resistance)
Locally suppressive agents
Locally and systemically active agents

During the last 15 years, prevention strategies have primarily focused on methods either to suppress or eliminate the endogenous microflora or to prevent the acquisition of new microorganisms. These have included a number of techniques, ranging from total protected isolation to the total suppression of the gastrointestinal microbial reservoir or the selective elimination of aerobic gram-negative organisms and preservation of the anaerobic flora to establish a "colonization resistance." The use of antibiotics to achieve systemic prophylaxis has also been studied both with and without simple reverse isolation. More recently, studies have begun to focus on methods to improve impaired host defenses, not only by treating the patient's underlying malignancy but also by trying to improve the phagocytic or immune defenses.

Prophylactic antibiotics have served as the cornerstone of infection prevention strategies aimed at the microbial flora; such prevention strategies include not only antibiotics but also drugs effective against fungi, parasites, and viruses (table 4). Some antibiotics, such as gentamicin, vancomycin, polymixin B, neomycin, amphotericin B, and colistin, are nonabsorbable and achieve their primary effect by the elimination of organisms contained within the alimentary tract. (Agents dispensed in a liquid formulation will affect the microbial flora of the entire alimentary canal, whereas those given as capsules or tablets will exert their activity only along the intestinal tract.) Other antibiotics, such as

TABLE 4.—Antimicrobial agents used for prophylaxis in cancer patients

Antibiotics	Antifungals	Antiparasitics	Antihelminths	Antivirals
Gentamicin (oral)	Nystatin	Trimethoprim-sulfamethoxazole	Thiabendazole	Acycloguanosine
Vancomycin (oral)	Amphotericin B (oral)			
Polymixin B (oral)	Miconazole			
Neomycin	Ketoconazole			
Framycetin				
Colistin				
Nalidixic acid				
Trimethoprim-sulfamethoxazole				
Erythromycin				

nalidixic acid or trimethoprim-sulfamethoxazole, not only alter the intestinal flora but also achieve therapeutic blood levels and hence may exert a systemic prophylaxis as well. Because of the cost and complexity of some of these preventive strategies, their utility in cancer patients must be critically assessed before they are accepted into common medical practice. Accordingly, a series of criteria, or "yardsticks," should be considered for each of the prophylactic regimens (table 5). For example, one must determine not only how effective the regimen is in preventing infectious morbidity and mortality but also whether the reduced incidence of infection permits the delivery of chemotherapeutic regimens which are effective in treating the underlying malignancy. In addition to efficacy, one must determine how tolerable the regimen is and whether compliance influences outcome. The side effects of the regimen, particularly the emergence of resistant organisms and fungal superinfections, must be carefully assessed. The risk for drug-induced organ toxic effects, such as myelosuppression, must also be considered along with the actual cost of the prophylactic regimen. In this discussion, the objective will be to offer a critical, although admittedly biased, review of these various prevention strategies, to determine what has been learned, thus permitting us to prioritize our present and future research objectives and treatment programs.

TABLE 5.—Yardsticks for assessing prophylactic antibiotics

EFFICACY
1. How effective is the regimen in decreasing infection-related morbidity and mortality?
2. Does the reduced incidence of infection permit delivery of more cancer chemotherapy?

COMPLIANCE
1. How tolerable is the prophylactic regimen?
2. Does compliance affect efficacy?

SIDE EFFECTS
1. What is the risk of emergence of resistant organisms?
2. What is the risk of superinfection?
3. What is the risk of organ-related toxicity (eg, myelosuppression, altered drug absorption)?

COST
1. Is the regimen cost-effective?

In many ways, the total protected environment has served as a paradigm for many of the infection-prevention regimens. The goal of this regimen is to suppress endogenous microorganisms while also preventing acquisition of new exogenous microorganisms. This necessitates a rather complicated and costly program which includes physical isolation in a HEPA (high-efficiency particulate air)-filtered laminar air flow room as well as alimentary canal or gastrointestinal decontamination with nonabsorbable antibiotics and cutaneous and mucosal antisepsis (18–20). A major problem with this approach has been poor compliance, usually due to the unpleasant taste of the nonabsorbable antibiotics. In a recent National Cancer Institute (NCI) study (21), 92% of the patients were initially taking the nonabsorbable antibiotics, but < 50% of the patients faithfully continued taking them after the first 2 weeks of isolation. This is not a trivial matter, since rebound infections with rapidly proliferating organisms such as *Pseudomonas aeruginosa* have been observed as the rule rather than the exception when prophylactic nonabsorbable antibiotics are suddenly discontinued while the patient remains granulocytopenic (22). Moreover, in our studies, the incidence of serious infection was increased in patients who were poorly compliant when compared to those patients who consistently took the prophylactic antibiotics. Total microbial suppression of the intestinal tract may also lead to other side effects of concern, including decreased absorption of nutrients or drugs such as methotrexate, and promotes an ideal setting for emergence of antibiotic-resistant bacteria acquired from the hospital environment. In spite of these liabilities, it is important to note that nearly every study performed during the last decade has confirmed that total protected isolation significantly reduces the incidence of serious infections in patients who have protracted periods of profound granulocytopenia. Nonetheless, the ultimate efficacy of this regimen must be balanced against

its high cost and side effects. In this regard, it is important to note that even total protected isolation does not reduce the incidence of infection to zero, but rather to approximately 5%–25% of that observed in a nonisolated setting (23,24). More importantly, the true value of this type of isolation is primarily observed in patients who have very protracted periods of granulocytopenia, usually exceeding 20 days of granulocyte counts < 100/μl, making it of merit for only a minority of patients (eg, such as those receiving very intensive chemoradiotherapy regimens or those undergoing bone marrow transplantation). Accordingly, total protected isolation has relatively restricted applicability for most cancer patients undergoing current induction or maintenance therapeutic regimens. For these patients, simpler alternative regimens have been sought.

Since the alimentary tract is recognized as an important reservoir of potential pathogens, total gastrointestinal or total alimentary tract decontamination without physical isolation has also been investigated. The antibiotics used for total decontamination are similar to those utilized in the total protected environment, and various studies have additionally included skin care, low microbial diets, and other hygienic programs. To date, however, the efficacy of nonabsorbable antibiotics alone has not been consistently proven; of the more than nine randomized clinical trials which have been conducted during the past decade, only three have demonstrated a significant reduction in infections in treated patients compared to untreated controls (24,25). While the reasons for these variable results are not entirely clear, a number of issues must be considered. Compliance in taking these antibiotics is often poor and has been accompanied by a significant risk of both rebound overgrowth of potential pathogens and acquisition of aminoglycoside-resistant strains from the hospital environment (26,27). No benefit has been demonstrated when such regimens have been utilized for patients with short periods of granulocytopenia or for those with only moderate (> 100 cells/μl) degrees of neutropenia. Often the oral cavity and esophagus have not been included for microbial suppression, leaving both a reservoir for intestinal recontamination and a source of potential pathogens to induce periodontitis, pharyngitis, and most importantly, pneumonitis (24). In addition, these antibiotics are very costly, averaging approximately $150/day/patient, further limiting their value. Hence, nonabsorbable antibiotics for total gastrointestinal or total alimentary tract decontamination also have limited utility and are not recommended except for patients who will undergo prolonged, profound granulocytopenia and be cared for in a total protected environment (19,20). In light of these problems, attention has turned to other antibiotics to achieve selective antimicrobial modulation while preserving resistance to colonization, to exert systemic prophylaxis, or both.

TABLE 6.—Rationale for colonization resistance

DEFINITION	Threshold needed to colonize conventional mice (eg, 10^9 *Escherichia coli*) compared to that needed to colonize germ-free animals ($0.5–1.0 \times 10^2$ *E. coli*)
BASIS	Preservation of intact anaerobic intestinal flora
MODULATION	Effect of antibiotics on aerobic vs anaerobic intestinal flora Antibiotics such as trimethoprim-sulfamethoxazole, nalidixic acid, polymixin B, and amphotericin B preserve the anaerobic flora, ie, help colonization resistance.

The concept of colonization resistance is based upon a series of elegant studies performed by van der Waaij and colleagues (28–30) in The Netherlands, in which they demonstrated that much larger oral inocula of aerobic organisms were necessary to colonize a conventional mouse than to colonize specific pathogen-free rodents (table 6). This resistance to colonization was found to reside, in part, in the preservation of an intact anaerobic intestinal flora, raising the possibility that certain antibiotics might be able to suppress the common pathogenic aerobes while preserving the microenvironment needed to maintain resistance to colonization with newly acquired organisms. This concept assumes potential practical importance for the neutropenic patient because infections in this setting are only very rarely due to anaerobes. Thus, suppression of the aerobic flora with preservation of the anaerobic flora might prove beneficial in infection prophylaxis while concurrently reducing the risk of acquiring a resistant aerobe, as was so frequently encountered with attempts at total suppression of alimentary canal flora.

Studies have now shown that antibiotics such as trimethoprim-sulfamethoxazole, nalidixic acid, polymixin B, oxolinic acid, and amphotericin B can eliminate aerobes and yeasts without affecting anaerobes. Having demonstrated the usefulness of these agents in providing colonization resistance and in decreasing the incidence of infections in experimental animals, a number of clinical trials have been undertaken. The first of these studies was performed by Hughes and colleagues (31) at St. Jude Children's Research Hospital, Memphis, TN, where trimethoprim-sulfamethoxazole was used in a randomized prospective trial to prevent *Pneumocystis carinii*. The study was not designed for bacterial infection prophylaxis, but upon analyzing the results of this trial, Hughes observed not only a near total absence of pneumocystis pneumonia but also a significant decrease in the incidence of bacterial infections, particularly those related to the upper respiratory tract and urinary tract. These observations prompted a number of additional studies, one of the first of which, reported by Gurwith and colleagues (32) from Manitoba, Canada, was in adults with acute nonlymphocytic leukemia. In this study, 41

patients were randomized to receive either trimethoprim-sulfamethoxazole or no prophylaxis following the administration of induction chemotherapy, and a significant reduction of bacteremia was observed in the trimethoprim-sulfamethoxazole-treated patients. Unfortunately, only a few patients with acute leukemia and profound neutropenia were studied, but these encouraging results led to a number of additional trials. Sleijfer et al (33) utilized trimethoprim-sulfamethoxazole or nalidixic acid (depending upon initial stool culture susceptibility results) with or without colistin (for those colonized with *Pseudomonas aeruginosa*) and compared this regimen to no prophylaxis among > 100 patients with marked neutropenia. Bacterial infections, especially gram-negative bacteremia, were very substantially reduced. In light of this positive experience, studies in the United States have attempted to simplify the colonization-resistance regimen by using single drugs (table 7). The first of these studies was conducted by Wade et al (34) and compared trimethoprim-sulfamethoxazole plus nystatin to a standard total gastrointestinal decontamination regimen utilizing gentamicin and nystatin. Although this study showed these two regimens to be comparable, multiple, severe infections occurred both in the trimethoprim-sulfamethoxazole-treated patients and in those who had undergone total alimentary canal decontamination. Hence, comparability between these regimens should not be taken to indicate true efficacy, especially in light of the inconsistent results which have been observed in prior studies with total gastrointestinal decontamination. The patients chosen for investigation were those with relapsed acute leukemia who could be expected to have severe mucosal damage and prolonged marrow aplasia, ie, those who were at exceedingly high risk of severe infections and hence provided a very demanding test of efficacy. Nonetheless, it is the opinion of the University of Maryland Cancer Center group that there is reduction in infections if the aerobic flora is completely suppressed. A second study by Wade et al (35), comparing trimethoprim-sulfamethoxazole to nalidixic acid, also appeared to be of limited efficacy but did demonstrate the development of resistance to both trimethoprim-sulfamethoxazole and nalidixic acid and a significant prolongation of neutropenia in patients receiving trimethoprim-sulfamethoxazole. A number of subsequent studies examining antibiotic prophylaxis have yielded conflicting results, thus clouding the benefit of trimethoprim-sulfamethoxazole monoprophylaxis (36–38). The reason for these disparate results may be related to variations in study design, the types of patients being treated, the concomitant use of other preventive measures used in concert with the prophylactic antibiotics, or even some unanticipated variables. One such confounding factor has been observed in a recent trial completed at the NCI (39). Patients who had completed a course of chemotherapy were randomized to receive either prophylactic antibiotics or placebos in a double-blind trial. In this study, we combined trimethoprim-sulfamethoxazole with erythromycin, not to maintain colonization resistance but to provide systemic prophylaxis against both gram-negative organisms and the increasing number of gram-positive bacteria we have recently encountered at the NCI (40).

TABLE 7.—Standard and modified regimens for achieving colonization resistance or selective antimicrobial modulation

SAM regimen
Nalidixic acid: 4–8 g/day
Trimethoprim-sulfamethoxazole: 320–480 mg/day
Polymixin B: 400–800 mg/day
Amphotericin B: 1–2 g/day
Modified colonization resistance
Trimethoprim-sulfamethoxazole + nystatin
(vs gentamicin + nystatin)
vs
Nalidixic acid + nystatin

During a $2^1/_2$-year period, 150 patients were randomized to receive either the combination of trimethoprim-sulfamethoxazole plus erythromycin or placebos (table 8) (39). Once randomized, patients continued to receive the same antibiotics or placebos during subsequent episodes during which they were eligible for study. This study was conducted in a young patient population whose median age was 17 years and whose underlying malignancies included acute leukemia (33%), non-Hodgkin's lymphoma (17%), and solid tumors (50%). As part of the study design, patients were prospectively evaluated for their compliance in taking either the drugs or the placebos and were rated as: 1—excellent, meaning that they took all of the medications or placebos during the period of risk; 2—good, in which case they missed no more than two consecutive doses or 24 hours' worth of antibiotics during the period that they were neutropenic; or 3—poor, in which case they missed > 24 hours' worth of medications or placebos. It is interesting to note that in spite of the commitment of these patients to enter a study which might keep them out of the hospital and the careful instructions they received about the importance of taking medications, only 70% complied in taking most of the prescribed medications. The importance of compliance as a variable is evidenced by the fact that in this study there is no difference in the incidence of fever or infection between trimethoprim-sulfamethoxazole or placebo groups if compliance is not considered (table 9). However, if the data are analyzed according to compliance, a significant decrease in the incidence of fever and infection is observed in trimethoprim-sulfamethoxazole-treated patients with excellent compliance. Importantly, this difference disappears for patients who had less than excellent compliance. Even more strikingly, compliance not only expresses an im-

TABLE 8.—NCI systemic antibiotic prophylaxis study

	Randomization groups		
	Trimethoprim-sulfamethoxazole + erythromycin	Placebo	Totals
No. of patients	77	73	150
No. of episodes	249	292	541
Median age (yrs)	16	17	17

portant dependent variable in this study but also seems to be a significant independent variable. Patients randomized to the drug group, who had excellent compliance, did significantly better compared to those with either good or poor compliance. The same difference is also observed in the placebo group, since placebo-treated patients with excellent compliance had a significant decrease in the incidence of fever or infection compared to those with less than excellent compliance. It is unclear what compliance was measuring in this study, but these data do suggest that other variables which could not be anticipated in the design of this clinical trial, such as patient diet, hygiene, or attitude, might have had a significant impact on the incidence of infectious complications. In addition, other modifying factors also appear to have had a role in the outcome of this trial, including the patient's underlying malignancy and the type of chemotherapy being administered (table 10) (40).

Overall, it appears that regimens which include trimethoprim-sulfamethoxazole as either the major or sole component of prophylaxis may offer some benefit in reducing the incidence of fever or infections in neutropenic cancer patients, although this does not appear to be as great as was observed in earlier studies and may be restricted to only certain patient populations. These limited benefits must be carefully balanced against the potential liabilities of trimethoprim-sulfamethoxazole prophylaxis which include prolongation of neutropenia in patients treated with higher doses of trimethoprim-sulfamethoxazole and the emergence of trimethoprim-sulfamethoxazole-resistant organisms and infections (35,36,39,41). Of particular importance is the recent observation of Wilson and Guiney (42) that resistance to trimethoprim-sulfamethoxazole may be plasmid-mediated and encode resistance to other antibiotics (eg, ticarcillin and ampicillin), thus posing an obvious threat to the therapeutic armamentarium used in the management of serious infections in immunosuppressed patients.

A neglected factor in many evaluations relates to the concurrent use of other agents which may reduce the alimentary canal anaerobes. Once these organisms are also suppressed, colonization resistance is diminished and the acquisition of antibiotic-resistant potential pathogens from the environment can be expected, just as it was found to occur with total microbial suppression in previous years. The problem may relate to either concurrent agents used as prophylaxis (eg, erythromycin) or other systemic agents given during periods of fever (eg, carbenicillin or related compounds which readily suppress the intestinal anaerobes) (43). Certainly, use of trimethoprim-sulfamethoxazole or any

TABLE 9.- **NCI STUDY: Overall Efficacy of Antibiotic Prophylaxis**

	Episodes with Fever or Infection	
	T/S + E*	Placebo
All Evaluable Episodes	49/222 (22.1%)	71/264 (26.7%)
Initial Episode per Patient	17/59 (28.8%)	21/60 (35.0%)
Episodes with Documented Granulocytopenia and:		
Total Compliance	19/105 (18.1%) — p = 0.009 — 46/143 (32.2%)	
	p = 0.05	p = 0.253
Partial Compliance	P < 0.001 — 9/25 (36.0%) — ns — 7/16 (43.8%)	p < 0.001
	p = 0.018	p = 0.006
Poor Compliance	18/26 (69.2%) — ns — 9/9 (100%)	

*T/S + E = trimethoprim-sulfamethoxazole + erythromycin.

TABLE 10.—NCI study: efficacy according to underlying malignancy and therapy for patients with granulocytopenia and total protocol compliance

	Episodes with fever or infection	
	Trimethoprim-sulfamethoxazole + erythromycin	Placebos
Underlying malignancy		
Leukemia*	5/32 (15.6%)	12/31 (38.7%)
Lymphoma	5/28 (17.9%)	12/41 (29.3%)
Solid tumors	9/45 (20.0%)	22/71 (31.0%)
Chemotherapy dosage		
Conventional†	16/92 (17.4%)	41/134 (30.6%)
Intensive	3/13 (23.1%)	5/9 (55.6%)
Treatment schedule		
Induction	8/32 (25.0%)	15/38 (39.5%)
Maintenance‡	11/73 (15.1%)	31/105 (29.5%)

*P = 0.037 (Fisher exact test).
†P = 0.027 (Fisher exact test).
‡P = 0.018 (Fisher exact test).

similar approach demands use of concurrent surveillance cultures to observe for resistant species (44). Hence, while trimethoprim-sulfamethoxazole prophylaxis is well-tolerated and inexpensive, it is not a prophylaxis panacea and it should be utilized in only limited circumstances under controlled conditions of intense supervision.

While these various studies suggest that antibiotics are unlikely to provide a totally effective prophylaxis, one must also consider the possibility that the limited benefits observed to date may be a reflection of the spectrum and potency of the antibiotics themselves. This caveat emanates in part from studies that Pizzo et al (40,45–47) have performed at the NCI over the last several years, in which iv antibiotics were used for systemic prophylaxis as well as therapy. These studies have shown that broad-spectrum systemic antibiotics may prevent second infections in febrile patients who remain neutropenic for protracted periods of time, regardless of whether their initial condition was due to a microbiologically documented infection or whether they had unexplained fever. These studies suggest, therefore, that if the right antibiotics and antifungal agents are available, more effective prophylaxis might be feasible.

Such a deficiency in antimicrobial efficacy is especially apparent for the prevention of fungal infections in cancer patients. During recent years, evidence of invasive fungal disease has been found in 8%–60% of patients dying with cancer and has prompted a number of studies employing specific antifungal prophylaxis. However, clear efficacy has not been convincingly demonstrated to date. Drugs like nystatin or amphotericin B, although commonly employed, may not be very effective (48,49). Increased interest has recently been given to the imidazoles, particularly ketoconazole, since it is orally absorbed and has a broad antifungal spectrum and a low level of toxicity. Although a number of studies are in progress, none has convincingly demonstrated a significant reduction of systemic fungal disease. Of additional concern, several studies have suggested that while ketoconazole may reduce colonization with *Candida* species, patients may nevertheless become colonized with *Torulopsis* and *Aspergillus*, organisms which are generally resistant to ketoconazole (50,51). It seems unlikely that ketoconazole will be the broad-spectrum antifungal panacea so clearly needed. Oral amphotericin B as lozenges or tablets or mixed with Orabase and applied to the gingiva may be the most effective antifungal agent available, presumably because there is a prolonged release of amphotericin B into the saliva in concentrations effective against the commonly infecting yeasts (52). Unfortunately, no comparative controlled trials have been performed, and these preparations of amphotericin B are not available in the United States.

In addition to studies focusing on bacteria and fungi, prevention of parasitic and viral infections has also received attention. Indeed, the prevention of *Pneumocystis carinii* by trimethoprim-sulfamethoxazole stands as perhaps the most convincing example of effective antimicrobial prophylaxis in the cancer patient (31). Nonetheless, *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole need not be given to every cancer patient, since the distribution and incidence of *Pneumocystis* are highly variable; it seems worthwhile to reserve prophylaxis for patients in centers where the incidence is high, especially in light of the potential side effects associated with trimethoprim-sulfamethoxazole.

Although data are lacking, another infection for which

prophylaxis might be beneficial is strongyloidiasis. *Strongyloides stercoralis* is a nematode which resides in the intestinal tract of patients from tropical climates and which undergoes postchemotherapy differentiation from its rhabditiform stage to an invasive filariform, the latter migrating through the intestinal wall, carrying with it various species of gastrointestinal bacterial pathogens. Anticipation of this hyperinfection syndrome by identification of the patient at risk and treatment of that patient with thiabendazole may prevent the life-threatening complication associated with this infection.

An exciting development in recent years has been the prospect of preventing serious viral infections. This is evidenced from the recent observation of Saral and colleagues (53) in which acycloguanosine was used to temporarily prevent herpes simplex infections in patients undergoing allogeneic bone marrow transplantation. Unfortunately, similar success has not been achieved against CMV, and reports of resistance developing to acycloguanosine have recently been published (54,55). Studies combining antiviral agents and interferons to prevent CMV pneumonitis are underway.

On the basis of these observations, the following general conclusions can be drawn concerning prophylactic antibiotic regimens:

(*a*) While studies have demonstrated that total protective isolation is effective in preventing infections in patients with protracted granulocytopenia, its cost and cumbersomeness limit its utility to selected patients—perhaps to those undergoing bone marrow transplantation or other similar degrees of intense, prolonged marrow aplasia.

(*b*) Total alimentary canal microbial suppression may be efficacious, but poor compliance with resultant inadequate suppression, rebound overgrowth when antibiotics are discontinued prematurely, and the risk of aminoglycoside-resistant organism acquisition and infection make this technique applicable only in the setting of the total protected environment.

(*c*) Selective antimicrobial modulation to suppress pathogenic aerobes while preserving colonization resistance is theoretically promising and early studies have shown clinical benefit, making this an area which deserves additional study.

(*d*) Systemic antimicrobial prophylaxis can be effective, but this depends upon the kinds of drugs which are being utilized. It is not clear what the relative contributions of systemic effect vs local suppression are at this time. Although trimethoprim-sulfamethoxazole has been used most commonly, its efficacy is not dependable in all clinical settings and there are potential side effects which may well outweigh its benefits in specific institutions. Nevertheless, the concept deserves further consideration.

(*e*) We would re-emphasize that any antibiotic prophylaxis should not be utilized alone without careful attention to those other practices, such as handwashing, low microbial foods and beverages, good housekeeping, etc. These are procedures which can be accomplished at any center at little or no added expense but unfortunately are often neglected.

In addition to regimens targeted against the microbe, methods to improve basic host defenses need careful consideration. Recent studies have included leukocyte transfusions, immunomodulators, active and passive immunization, and nutritional support. While leukocyte transfusions are theoretically appealing, their efficacy has not been substantiated (56) by individual studies (57–59) or by a large multi-institution study (60) (table 11), probably due to technical limitations in preparing

TABLE 11.—Prophylactic granulocyte transfusions in acute myelogenous leukemia (cooperative trial)*

	Documented infections	
	Transfusion	Control
No. of patients	54	48
No. with any infection	25 (46)	20 (42)
Bacterial septicemia (episodes)	5 (9)	13 (27)
Gram-negative	3 (6)	8 (17)
Gram-positive	2 (4)	8 (17)
Fungal septicemia (episodes)	3 (6)	1 (2)
Pneumonia (episodes)	12 (22)	6 (13)
Bacterial	2 (4)	0 (0)
Fungal	2 (4)	2 (4)
Unknown	8 (15)	4 (8)
Cellulitis/abscess	8 (15)	7 (14)
Urinary tract (episodes)	3 (6)	1 (2)
Total episodes of infection	28	31

**From* ref 60.

sufficient quantities of leukocytes with adequate functional quality to provide effective replacement of the cancer patients' limited granulocyte reserves. In addition, side effects including fever, chills, pulmonary infiltrates, and especially alloimmunization severely limit the applicability of this modality. A possible exception may be the use of granulocytes following bone marrow transplantation, provided the donor of the marrow is also the granulocyte donor (61).

Another approach is use of immunomodulators to accelerate granulocyte recovery following chemotherapy. The first of these to be used was BCG, while more recent studies have focused on lithium carbonate. While lithium may shorten the duration of moderate (100–500 cells/μl) neutropenia in patients with acute leukemia and small cell lung cancer, there is no change in the period of profound (< 100 cells/μl) granulocytopenia. This is to be expected because lithium mobilizes granulocytes from the bone marrow but does not speed their maturation. Thus, the patient with an aplastic marrow has no granulocytes to release under stimulation with lithium. Not surprisingly, therefore, a significant reduction in the incidence of infections has not been convincingly demonstrated (62). Moreover, one must recognize that lithium has a number of metabolic and renal side effects and may even have oncogenic potential. It must be noted, however, that the study of biologic response modifiers is in its infancy, and an increasing number of chemically defined immunoregulatory agents are currently being developed, making this a likely area for future investigation.

In addition to improving phagocytic defenses, investigations have also focused on humoral and cellular immunity. Such studies generally reside in either active or passive immunization. Probably as a consequence of immunosuppression, however, active immunization has been disappointing in patients receiving cytotoxic therapy, since effective antibody titers have not been sustained with the bacterial vaccines against *Pneumococcus* and *Streptococcus pneumoniae* (63–65). The influenza virus vaccine may produce adequate antibody levels to assure protection, provided the vaccine is given some days either before or after chemotherapy (66). Although influenza is no more common among cancer patients than any others, the major concern relates to possible bacterial superinfection during the myelosuppressed state. Therefore, the influenza vaccine should be considered routinely. Another possible exception to this might be the current varicella-zoster vaccine (67). However, studies with this live virus vaccine have yet to be carried out in a patient receiving more intensive chemotherapy (such as during induction), and enthusiasm must be reserved until safety is more clearly demonstrated in more severely immunocompromised patients.

Attention has also recently focused on passive immunization as a means for achieving therapeutic antibody titers in immunosuppressed patients, particularly because of the recent feasibility of administering gamma globulins iv. Along these lines are the studies of the San Diego group (68) on the use of antibody directed at the core glycolipid of the *Enterobacteriaceae*. This "J5 antiserum" has shown promising results in experimental animal studies and clinical trials, so that further investigations of the use of this antiserum as an adjunct to both therapy and prevention would be appropriate (69,70).

Finally, in addition to these more conventional routes of infection prevention, attention has also been directed at trying to improve the nutritional status of cancer patients to maintain or enhance their host defense systems. For example, we recently randomized patients undergoing intensive chemotherapeutic regimens to receive either parenteral hyperalimentation or standard nutrition and asked whether this might influence the duration of myelosuppression in these patients (71). While patients in this study had a significantly higher caloric and protein intake if they received hyperalimentation, we observed no difference in the duration of myelosuppression in the two groups of patients. The number of patients available for study was small, however, making additional trials worthwhile.

While still in their early days, methods to improve host defenses as an adjunct to infection prevention clearly merit additional investigation.

It is clear that both considerable gains and considerable failures have been realized over the past decade as various methods of infection prevention have been explored. While it is tempting to continue to utilize certain modalities simply because it seems better to do something than nothing, it is essential that we keep a very critical and focused view on the benefits and liabilities of each of the methods that are currently being employed. At this time, it appears that effective prevention is unlikely to be accomplished from any single approach but rather is likely to require multiple modalities, probably combining antibiotic prophylaxis, simple approaches to reduce organism acquisition, and host-oriented prevention strategies.

REFERENCES

1. Schimpff SC, Young VM, Greene WH, et al. Origin of infection in acute nonlymphocytic leukemia: significance of hospital acquisition of potential pathogens. Ann Intern Med 77:707, 1972.
2. Johanson WG, Pierce AK, Sanford JP, et al. Changing pharyngeal flora of hospitalized patients: emergence of gram-negative bacilli. N Engl J Med 281:1137, 1969.
3. ———. Nosocomial respiratory infections with gram-negative bacilli: the significance of colonization of the respiratory tract. Ann Intern Med 77:701, 1972.
4. Fainstain V, Rodriguez V, Turk M, et al. Patterns of oropharyngeal and fecal flora in patients with leukemia. J Infect Dis 144:10, 1981.

5. Remington JS, and Schimpff SC. Please don't eat the salads. N Engl J Med 304:433, 1981.
6. Pizzo PA, Waters CW, Purvis D, et al. Microbiological evaluation of food items for patients undergoing gastrointestinal decontamination and protected isolation. J Am Diet Assoc 81:272, 1982.
7. Aisner J, Schimpff SC, Bennett JE, et al. Aspergillus infection in cancer patients: association with fireproofing materials in new hospitals. JAMA 235:411, 1976.
8. Leclair JM, Zaia J, Levin MJ, et al. Airborne transmission of chickenpox in a hospital. N Engl J Med 302:450, 1980.
9. Young LS. Nosocomial infections in the immunocompromised adult. Am J Med 70:398, 1981.
10. Hughes WT, and Townsend TR. Nosocomial infections in immunocompromised children. Am J Med 70:412, 1981.
11. Albert RK, and Condie R. Handwashing patterns in medical intensive care units. N Engl J Med 304:1465, 1981.
12. Schimpff SC, Greene WH, Young VM, et al. Significance of *Pseudomonas aeruginosa* in the patient with leukemia or lymphoma. J Infect Dis 130:524, 1974.
13. Wingard JR, Merz WG, Saral R, et al. *Candida tropicalis:* a major pathogen in immunocompromised patients. Ann Intern Med 91:539, 1979.
14. Pizzo PA. Infectious complications in the child with cancer: I. Pathophysiology of the compromised host and the initial evaluation and management of the febrile cancer patient. J Pediatr 98:341, 1981.
15. ———. Infectious complications in the child with cancer: II. Management of specific infectious organisms. J Pediatr 98:513, 1981.
16. Schimpff SC, O'Connell MJ, Greene WH, et al. Infections in 92 splenectomized patients with Hodgkin's disease. A clinical review. Am J Med 59:695, 1975.
17. Winston DJ, Gale RP, Meyer DV, et al. Infectious complications of human bone marrow transplantation. Medicine 58:1, 1979.
18. Schimpff SC. Laminar air flow room reverse isolation and microbial suppression to prevent infection in patients with cancer. Cancer Chemother Rep 59:1055–1060, 1975.
19. Pizzo PA. Do results justify the expense of protected environments? *In* Controversies in Oncology (Wiernik PH, ed). New York, John Wiley and Sons, 1981, p 267.
20. Schimpff SC. Protective environments for the treatment of high risk cancer patients. *In* Controversies in Oncology (Wiernik PH, ed). New York, John Wiley and Sons, p 247.
21. Pizzo PA. Protected isolation of pediatric cancer patients receiving intensive chemotherapy: influence of autologous bone marrow rescue on infectious complications. *In* Proceedings of the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1981, p 799.
22. Schimpff SC, Greene WH, Young VM, et al. Infection prevention in acute nonlymphocytic leukemia: laminar air flow room reverse isolation with oral, nonabsorbable antibiotic prophylaxis. Ann Intern Med 82:351, 1975.
23. Bodey GP, and Rodriguez V. Infections in cancer patients on a protective environment-prophylactic antibiotic program. Am J Med 59:497, 1975.
24. Schimpff SC. Infection prevention in patients with cancer and granulocytopenia. *In* Infections Complicating the Abnormal Host (Grieco MH, ed). New York, Yorke Medical Books, 1980, p 926.
25. Pizzo PA. Antibiotic prophylaxis in the immunosuppressed patient with cancer. *In* Current Clinical Topics in Infectious Diseases, 4th ed (Remington JS, and Swartz MN, eds). New York, McGraw-Hill. In press.
26. Klastersky J, Debusscher L, Weerts D, et al. Use of oral antibiotics in protected environment unit: clinical effectiveness and role in the emergence of antibiotic resistant strains. Pathol Biol 22:5, 1974.
27. Hahn DM, Schimpff SC, Young VM, et al. Amikacin and cephalothin: an empiric regimen for granulocytopenic cancer patients. Antimicrob Agents Chemother 12:618, 1977.
28. van der Waaij D, Berghuis-De Vries JN, Lekkerkerk-van der Wees JEC, et al. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J Hyg (London) 69:405, 1971.
29. ———. Determination of the colonization resistance of the digestive tract of individual mice. J Hyg (London) 72:379, 1974.
30. ———. Selective elimination of Enterobacteriaceae species from the digestive tract in mice and monkeys. J Hyg (London) 72:205, 1974.
31. Hughes WT, Kuhn S, Chaudhary S, et al. Successful chemoprophylaxis for *Pneumocystis carinii* pneumonitis. N Engl J Med 297:1419, 1977.
32. Gurwith MJ, Brunton JL, Lank BA, et al. A prospective controlled investigation of prophylactic trimethoprim-sulfamethoxazole in hospitalized granulocytopenic patients. Am J Med 66:248, 1979.
33. Sleufer DT, Mulder NH, deVries-Hospers HG, et al. Infection prevention in granulocytopenic patients by selective decontamination of the digestive tract. Eur J Cancer 16:859, 1980.
34. Wade JC, Schimpff SC, Hargadon MT, et al. A comparison of trimethoprim-sulfamethoxazole plus nystatin with gentamicin plus nystatin in the prevention of infections in acute leukemia. N Engl J Med 304:1057, 1981.
35. Wade JC, deJongh CA, Newman KA, et al. A comparison of trimethoprim-sulfamethoxazole to nalidixic acid: selective decontamination as infection prophylaxis during granulocytopenia. J Infect Dis. In press.
36. Dekker A, Rozenberg-Arska M, Sixma JJ, et al. Prevention of infection by trimethoprim-sulfamethoxazole plus amphotericin B in patients with acute nonlymphocytic leukemia. Ann Intern Med 95:555, 1981.
37. Weiser B, Lange M, Fialk MA, et al. Prophylactic trimethoprim-sulfamethoxazole during consolidation chemotherapy for acute leukemia: a controlled trial. Ann Intern Med 95:436, 1981.
38. Zinner S, Gaya H, Glauser M, et al. Co-trimoxazole and reduction of risk of infection in neutropenic patients. A progress report for the EORTC Antimicrobial Therapy Project Group. *In* Proceedings of the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1981, p 795.
39. Pizzo PA, Robichaud KJ, Edwards BK, et al. Oral antibiotic prophylaxis in cancer patients: a double blind randomized placebo controlled trial. J Pediatr 102:125, 1983.
40. Pizzo PA, Robichaud KJ, Wesley R, et al. Fever in the pediatric and young adult patient with cancer. A prospective study of 1001 episodes. Medicine 61:153, 1982.
41. deJongh CA, Schimpff SC, Wiernik PH, et al. Resistant *E. coli* septicemia in leukemic patients receiving trimethoprim-sulfamethoxazole or nalidixic acid as prophylaxis. Ann Intern Med 95:555, 1981.
42. Wilson JM, and Guiney DG. Failure of oral trimethoprim-sulfamethoxazole prophylaxis in acute leukemia: isolation of resistant plasmids from strains of *Enterobacteriaceae* causing bacteria. N Engl J Med 306:16, 1982.
43. Hargadon MT, Young VM, Schimpff SC, et al. Selective suppression of alimentary tract microbial flora as prophylaxis during granulocytopenia. Antimicrob Agents Chemother 20:620, 1981.
44. Schimpff SC. Surveillance cultures. J Infect Dis 144:81–84, 1981.
45. Pizzo PA, Robichaud KJ, Gill FA, et al. Duration of empiric antibiotic therapy in granulocytopenic patients with cancer. Am J Med 67:194, 1979.
46. Pizzo PA, Ladisch S, Robichaud KJ, et al. Treatment of gram-positive septicemia in cancer patients. Cancer 45:206, 1980.
47. Pizzo PA, Robichaud KJ, Gill FA, et al. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. Am J Med 72:101, 1982.

48. Williams C. Oral anticandidal prophylaxis in patients undergoing chemotherapy for acute leukemia. Med Pediatr Oncol 3:275, 1977.
49. Ezdinli EZ, Icli F, Costello W, et al. Oral amphotericin for candidiasis in patients with hematologic neoplasms. JAMA 242:258, 1979.
50. Meunier-Carpentier F, Snoeck R, and Klastersky J. Oral antifungal prophylaxis in neutropenic cancer patients. *In* Proceedings of 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, 1982, p 10.
51. Acuna G, Winston DJ, Young LS, et al. Ketoconazole prophylaxis of fungal infections in the granulocytopenic patient: a double-blind randomized, controlled trial. *In* Proceedings of 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1981, p 852.
52. de Vries-Hospers HG, and van der Waaij D. Amphotericin B concentrations in saliva after application of 2% amphotericin B in Orabase®. Infection 6:16, 1978.
53. Saral R, Burns WH, Laskin OL, et al. Acyclovir prophylaxis of herpes simplex virus infections: a randomized double-blind controlled trial in bone marrow-transplant recipients. N Engl J Med 305:63, 1981.
54. Schnipper LE, and Crumpacker CS. Resistance of herpes simplex virus to acycloguanosine: role of viral thymidine kinase and DNA polymerase loci. Proc Natl Acad Sci USA 77:2270, 1980.
55. Crumpacker SC, Schnipper LE, Marlowe SI, et al. Resistance to antiviral drugs of herpes simplex virus isolated from a patient treated with acyclovir. N Engl J Med 306:343, 1982.
56. Schiffer CA. Is prophylactic granulocyte transfusion in the best interest of the patient? *In* Controversies of Oncology (Wiernik PH, ed). New York, John Wiley and Sons, 1981, p 233.
57. Ford JM, and Cullen MH. Prophylactic granulocyte transfusions. Exp Hematol 5:65, 1977.
58. Schiffer CA, Aisner J, Daly PA, et al. Alloimmunization following prophylactic granulocyte transfusion. Blood 54:766, 1979.
59. Mannoni P, Rodet M, Coquin-Radeau E, et al. Efficacy of prophylactic granulocyte transfusions in preventing infections in acute leukemia. Blood Trans Immunohaematol 22:503, 1979.
60. Strauss RG, Connett JE, Gale RP, et al. A controlled trial of prophylactic granulocyte transfusions during initial induction chemotherapy for acute myelogenous leukemia. N Engl J Med 305:597, 1981.
61. Clift RA, Sanders JE, Thomas ED, et al. Granulocyte transfusions for the prevention of infection in patients receiving bone-marrow transplants. N Engl J Med 298:1052, 1978.
62. Lyman GH, Williams CC, Preston D, et al. The use of lithium carbonate to reduce infection and leukopenia during systemic chemotherapy. N Engl J Med 302:257, 1980.
63. Young LS, Meyer RS, Armstrong D, et al. *Pseudomonas aeruginosa* vaccine in cancer patients. Ann Intern Med 79:518, 1973.
64. Pennington JE, Reynolds HY, Woods RE, et al. Use of *Pseudomonas aeruginosa* vaccine in patients with acute leukemia and cystic fibrosis. Am J Med 58:629, 1975.
65. Siber G, Weitzman SA, Aisenberg AC, et al. Impaired antibody response to pneumococcal vaccine after treatment for Hodgkin's disease. N Engl J Med 299:442, 1978.
66. Ortbals DW, Liebhaber H, Presant CA, et al. Influenza immunization of adult patients with malignant diseases. Ann Intern Med 87:552, 1977.
67. Gershon AA. Live attenuated varicella-zoster vaccine. Rev Infect Dis 2:393, 1980.
68. Glauser MD, McCutchan JA, Ziegler EJ, et al. Adjuvant therapy for severe infections. *In* Combination Antibiotic Therapy in the Compromised Host (Klastersky J, and Staquet M, eds). New York, Raven Press, 1982, p 239.
69. Ziegler EJ, McCutchen JA, Douglas H, et al. Prevention of lethal *Pseudomonas* bacteremia with epimerase-deficient *E. coli* antiserum. Trans Assoc Am Phys 88:101, 1975.
70. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gramnegative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. N Engl J Med 307:1225, 1982.
71. Shamberger RC, Pizzo PA, Goodgame JT, et al. The effect of total parenteral nutrition on chemotherapy induced myelosuppression: a randomized study. Am J Med 74:40, 1983.

Ip Cisplatin in Patients With Malignant Ascites: Pharmacokinetic Evaluation and Comparison With the Iv Route[1,2,3]

Ephraim S. Casper,* David P. Kelsen, Nancy W. Alcock, and John L. Lewis, Jr[4,5]

Ip chemotherapy may be useful in managing patients with malignant ascites. This approach should result in a high concentration of drug at the site of disease, while the plasma concentration and therefore systemic toxicity remain low. Six patients with malignant ascites were treated with cisplatin at a dose of 60 mg/m^2. Three patients received the drug iv; one of these patients and three other patients were treated with ip cisplatin. Platinum concentrations in plasma, ascites, protein-free ultrafiltrates of plasma and ascites, and urine were assayed by flameless atomic absorption spectrophotometry. The area under the concentration × time curve for ultrafilterable platinum in ascites was 30 times greater after ip administration of cisplatin than after iv cisplatin. Ip cisplatin can be administered safely to patients with malignant ascites and may be useful in patients with minimal residual intraperitoneal disease, particularly ovarian carcinoma. [Cancer Treat Rep 67:235–238, 1983]

Malignant effusions are frequently seen in patients with advanced cancer. Generally, these effusions respond to systemic antineoplastic therapy when such therapy is effective against the particular tumor. However, management of malignant pleural effusions and ascites in patients whose tumors are resistant to systemic agents is a common clinical problem.

Pharmacokinetic modeling suggests that ip administration of certain drugs will result in a greater concentration of such drugs in the peritoneal fluid than in the plasma (1). Cisplatin may be an important drug for ip use; it is active against a variety of human tumors, especially ovarian carcinoma (2). Cisplatin is generally nonirritating to human tissues and does not require hepatic metabolism for activation. Although cisplatin binds to plasma proteins, the active form of the drug is not bound and can be separated from plasma by ultrafiltration (3).

This pilot study had two major objectives: (*a*) to determine whether cisplatin can be administered safely by ip infusion and (*b*) to compare the pharmacokinetics of platinum in patients with ascites who are treated with cisplatin by iv infusion with platinum kinetics in patients who receive the drug ip.

PATIENTS, METHODS, AND MATERIALS

Six adult female patients with cytologically confirmed malignant ascites were treated in this study. Two of the patients had adenocarcinoma of the breast and four had advanced ovarian cancer. All of these patients had received prior chemotherapy. Each patient had a performance status of ≥ 60%, a creatinine clearance > 60 ml/min, and normal hepatic function. No chemotherapy or radiation therapy had been administered within 4 weeks of treatment, and all patients had a wbc count > 4000/mm^3 and a platelet count > 150,000/mm^3 at the time they were first treated in this study.

An Intracath radiopaque catheter (Deseret Co, Sandy, UT) was inserted into the peritoneal cavity of each patient by percutaneous puncture under local anesthesia. All patients received an iv infusion of 5% dextrose in one-half normal saline at the rate of 200 ml/hour for 2 hours before and 4 hours after their treatment. Cisplat-

[1]Received July 27, 1982; revised Dec 7, 1982; accepted Jan 13, 1983.

[2]Supported by the Paul Garrett Fund, and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Casper is the recipient of an American Cancer Society Junior Faculty Fellowship Award.

[3]Presented in part at the 18th annual meeting of the American Society of Clinical Oncology, St. Louis, MO, 1982.

[4]Department of Medicine (*E. S. Casper, D. P. Kelsen,* and *N. W. Alcock*), and the Gynecology Service, Department of Surgery (*J. L. Lewis*), Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.

[5]We thank Dr. Brian Leyland-Jones for his helpful advice and Mary Jane O'Rourke for her careful preparation of this manuscript.

**Reprint requests to:* Ephraim S. Casper, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

in (60 mg/m²) at a concentration of 1 mg/ml was administered by iv infusion over 15 minutes to three patients. One of these three patients and three other patients were subsequently treated with cisplatin by ip infusion. For the ip infusions, cisplatin (60 mg/m²) was diluted in 500 ml of normal saline and administered ip by gravity flow following removal of 500 ml of ascites by paracentesis. The duration of the ip infusion was 15–20 minutes. Thus, all patients received the same dose of cisplatin (60 mg/m²).

For each patient, blood and ascites were collected in evacuated heparinized tubes (Becton-Dickinson Vacutainer 3200 KA, Becton-Dickinson and Co, Rutherford, NJ). Samples were taken before cisplatin administration and at 5, 10, 15, and 30 minutes and 1, 2, 4, 6, and 24 hours after the infusion ended. At 24 hours, paracentesis was initiated at the rate of approximately 200 ml/hour. Ascites, if present, and blood were sampled at 24-hour intervals thereafter for 3 days. Plasma was separated from whole blood by centrifugation. Plasma and ascites were filtered through Centriflo CF-25 conical filters (Amicon Corp, Lexington, MA) to separate protein-unbound platinum species. Urine was sampled before treatment and serial 24-hour pools of urine were collected for 3–5 days following the cisplatin infusion.

The concentration of elemental platinum in samples of plasma, ascites, ultrafiltrates of plasma and ascites, and urine was assayed by flameless atomic absorption spectrophotometry using a modification (4) of the method of Pera and Harder (5). Recovery of platinum added to pretreatment ascites, plasma, and urine did not differ significantly from 100%.

Patients were followed with daily serum creatinine determinations for 3–5 days after cisplatin administration and then once weekly for 3 weeks. Cbcs and physical examinations were also performed weekly for 3 weeks.

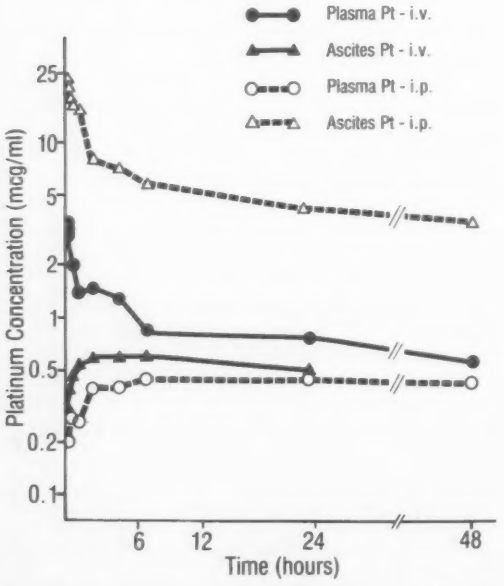


FIGURE 1.—Concentrations of platinum (Pt) in plasma and ascites after iv (solid lines) and ip (broken lines) administration of cisplatin (60 mg/m²).

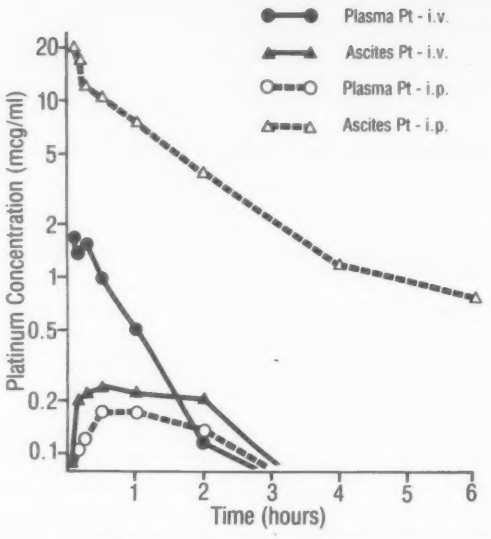


FIGURE 2.—Concentrations of ultrafilterable platinum (Pt) in plasma and ascites after iv or ip administraiton of cisplatin (60 mg/m²).

RESULTS

The mean sequential concentrations of total platinum in plasma and ascites after iv and ip administration of cisplatin are shown in figure 1. Initially, platinum is distributed rapidly from plasma after iv administration and from ascites after ip administration of cisplatin. A prolonged elimination phase is noted in both situations; the half-life for the elimination phase of total platinum from plasma after iv administration was 67 hours, and the half-life of the elimination phase for total ascites platinum after ip infusion was 33 hours.

The changes in ultrafilterable, non-protein-bound platinum are demonstrated in figure 2. After iv therapy, very little ultrafilterable platinum enters the peritoneal cavity, whereas high concentrations of ultrafilterable platinum are achieved and maintained in ascites after ip administration of cisplatin. At 24 hours following completion of the cisplatin infusion, the area

TABLE 1.—Platinum kinetics in patients treated with iv and ip cisplatin

Parameter	Route of administration	
	Iv	Ip
Maximum concentration (μg/ml)		
Ascites, total platinum	0.6	23.0
Ascites, ultrafilterable platinum	0.3	20.5
Plasma, total platinum	3.5	0.5
Plasma, ultrafilterable platinum	1.7	0.2
AUC at 24 hrs (μg/ml/hr)		
Ascites, total platinum	13.3	148.0
Ascites, ultrafilterable platinum	1.1	31.5
Plasma, total platinum	23.2	10.6
Plasma, ultrafilterable platinum	1.5	8.0
Urinary excretion at 24 hrs	41%	9%

under the concentration × time curve (AUC) for ultrafilterable platinum in ascites was 30 times greater after ip therapy than after iv therapy (table 1).

The pattern of urinary excretion of platinum varied according to the route of administration. At 24 hours following iv cisplatin administration, 41% of the administered platinum had been excreted in the urine, whereas urinary excretion 24 hours after ip therapy was only 9%.

Treatment was generally well-tolerated by all patients. Those patients who received iv cisplatin experienced the expected moderate nausea and vomiting. Three of the four patients who received ip cisplatin had only mild nausea; the other patient had moderate nausea and vomiting lasting 6 hours. No patient experienced a significant change in serum creatinine. Myelosuppression occurred in only one patient whose wbc and platelet counts fell to 3000 and 110,000/mm^3, respectively, 2 weeks after iv cisplatin. No patient had signs or symptoms suggestive of peritonitis.

One patient with ovarian cancer previously treated with cyclophosphamide, doxorubicin, and 5-FU experienced complete resolution of her ascites after one dose of ip cisplatin. This response was maintained with iv cisplatin for 5 months until she developed cutaneous metastases on her back, far from the paracentesis site. Her ascites, however, never recurred.

DISCUSSION

Although chemotherapy has been administered into body cavities for approximately 25 years (6), the optimal therapy for malignant effusions has not yet been defined.

Pleural effusions, even in patients with drug-resistant tumors, are often successfully treated by thoracentesis followed by intrapleural administration of an agent which causes inflammation of the pleural surfaces. This procedure results in adherence of the visceral to the parietal pleura, obliterating a potential space where fluid might reaccumulate.

The management of malignant ascites is more difficult. Induction of a chemical peritonitis may result in pain for the patient or perforation or obstruction of the bowel. A preferred strategy for managing this problem may be a direct attack with cytotoxic agents which do not irritate the peritoneal lining.

Malignant ascites may occur in many neoplastic conditions and occurs commonly in patients with ovarian carcinoma. This disease characteristically spreads within the peritoneal cavity. Even after extensive surgery or intensive chemotherapy, microscopic residual disease may persist.

Dedrick and co-workers (1) have calculated that after ip administration certain hydrophilic anticancer drugs would be expected to maintain a significantly greater concentration in the peritoneal cavity than in the plasma. Based on those calculations, clinical trials have been conducted using a peritoneal dialysis technique. The so-called "belly bath" has been performed using methotrexate (7), 5-FU (8), and doxorubicin (9). This method, which generally employs a surgically placed peritoneal catheter, can result in discomfort to the patient, and bacterial peritonitis may occur.

Cisplatin possesses therapeutic activity against ovarian cancer, even in patients having previously received multiple chemotherapeutic agents (2). A safe and simple technique for ip administration of cisplatin might have wide applicability. Krikorian et al (10) conducted a phase I study of ip cisplatin given in relatively low doses to patients with malignant ascites secondary to ovarian cancer. Although pharmacokinetic analyses were performed using total plasma and ascites platinum, these investigators did not examine the kinetics of the ultrafilterable, biologically active platinum species. Likewise, Lagasse and co-workers (11) performed pharmacokinetic studies in dogs treated with radiolabeled

cisplatin. Although these investigators demonstrated that the ip route results in higher drug levels in peritoneal tissues, they did not evaluate the kinetics of the active, ultrafilterable, platinum species. Belt et al (12) reported that after iv administration of cisplatin to two patients, filterable platinum levels in ascitic fluid rose quickly to an early peak about 30 minutes after completion of cisplatin administration. They also suggested that the ascitic fluid may serve as a reservoir for filterable platinum. Our findings are compatible with these data.

Our study included only patients who had malignant ascites. We studied the pharmacokinetics of ultrafilterable platinum species after both iv and ip administered cisplatin. Rather than using a permanent peritoneal dialysis catheter, we gave cisplatin as a single dose directly into the ascites. This approach permitted the drug to bathe the peritoneal surface while producing a minimum of patient discomfort. Neither special nursing nor long hospitalization was necessary.

Our data indicate that following ip administration of cisplatin, very high levels of active platinum species are achieved and maintained in ascites. These levels cannot be achieved in ascites by iv administration alone. In our patients who had abdominal cancer, however, it is probable that all areas of the peritoneal surface were not exposed to the same concentration of active platinum species (13). In addition, individual patients have different volumes of ascites with varying protein concentrations. Thus, the kinetics of platinum given to such patients may vary considerably between patients and within any given patient over a period of time.

The major potential for ip therapy with cisplatin may be in its use as an adjuvant following other therapeutic modalities in patients with ovarian cancer who have positive peritoneal cytology or no known residual disease. We are encouraged by the long-term control of malignant ascites in one of our two patients with advanced ovarian cancer. Ip chemotherapy with cisplatin is technically feasible and pharmacokinetically rational. Further trials, perhaps with the addition of protective agents (14), are warranted.

REFERENCES

1. Dedrick RL, Myers CE, Bungay PM, et al. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1–11, 1978.
2. Bruckner HW, Cohen CJ, Wallach RC, et al. Treatment of advanced ovarian cancer with *cis*-dichlorodiammineplatinum(II): poor-risk patients with intensive prior therapy. Cancer Treat Rep 62:555–558, 1978.
3. Holdener EE, Park CH, Belt RJ, et al. Effect of mannitol (MA) and human plasma (HP) on cytotoxicity of *cis*-diamminedichloroplatinum (DDP). Clin Res 26:436A, 1978.
4. Kelsen DP, Hoffman J, Alcock N, et al. Pharmacokinetics of cisplatin regional hepatic infusions. Am J Clin Oncol 5:173–178, 1982.
5. Pera MF, Jr, and Harder HC. Analysis for platinum in biological material by flameless atomic absorption spectrophotometry. Clin Chem 23:1245–1249, 1977.
6. Karnofsky DA, Abelman WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of carcinoma. Cancer 1:634–656, 1948.
7. Jones RB, Collins JM, Myers CE, et al. High-volume intraperitoneal chemotherapy with methotrexate in patients with cancer. Cancer Res 41:55–59, 1981.
8. Speyer JL, Collins JM, Dedrick RL, et al. Phase I and pharmacologic studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40:565–572, 1980.
9. Ozols RF, Young RC, Speyer JL, et al. Intraperitoneal (IP) adriamycin (ADR) in ovarian carcinoma (OC). Proc Am Assoc Cancer Res and ASCO 21:425, 1980.
10. Krikorian JG, Lopez JA, Reich SD, et al. Phase I study of intraperitoneal cis-platinum. Proc Am Assoc Cancer Res and ASCO 22:361, 1981.
11. Lagasse LD, Pretorius RG, Petrelli ES, et al. The metabolism of cis-dichlorodiammineplatinum (II): distribution, clearance and toxicity. Am J Obstet Gynecol 139:791–798, 1981.
12. Belt RJ, Himmelstein KJ, Patton TF, et al. Pharmacokinetics of non-protein-bound platinum species following administration of *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 63:1515–1521, 1979.
13. Dunnick NR, Jones RB, Doppman JL, et al. Intraperitoneal contrast infusion for assessment of intraperitoneal fluid dynamics. AJR 133:221–223, 1979.
14. Howell SB, Pfeifle CE, Wung WE, et al. Intraperitoneal chemotherapy with cisplatin (DDP). Proc ASCO 1:27, 1982.

Phase I Study of MVE-2 Therapy in Human Cancer[1,2]

Adan Rios,* Michael Rosenblum, Marvin Powell,† and Evan Hersh[3]

MVE-2, a polymer of maleic anhydride and divinyl-ether (molecular weight, 15,500), was given to 57 patients in a phase I study. The agent was selected for study because it was a potent macrophage activator, interferon inducer, and immunotherapeutic agent in animal tumor models. The drug was administered by iv infusion over a 1-hour period using three schedules of administration: (*a*) weekly at doses of 25–650 mg/m², (*b*) every other week at doses of 500–1200 mg/m², and (*c*) every 3 weeks at doses of 1200–1500 mg/m². No cardiac, pulmonary, hematologic, or hepatic toxicity was observed. There were 25 episodes of asymptomatic proteinuria in 26 patients who received MVE-2 dose levels of ≥ 500 mg/m². It was not associated with changes in BUN or creatinine. The proteinuria began approximately 4 weeks after the start of therapy and lasted approximately 4–6 weeks after the therapy was terminated. Proteinuria resolved in all patients followed. At present, proteinuria appears to be the major dose-limiting toxicity. None of the patients had a partial or complete response although there was evidence of biologic activity with measurable tumor regression in five patients. No major modification of host defense parameters was noted. Further studies should be directed towards determining the nature of the proteinuria and whether changes in the rate or schedule of administration can modify the proteinuria or increase the host defense modification. [Cancer Treat Rep 67:239–243, 1983]

MVE-2 is an anionic polymer of maleic anhydride and divinyl-ether with a molecular weight (MW) of 15,500. It is one of a family of five polymers of defined MW which have been prepared and which are referred to as MVE 1–5. They have molecular weights of 12,500, 15,500, 21,300, 32,000, and 52,600, respectively (1).

MVE-2 and its congeners were developed based on the observed biologic, immunologic, and antitumor properties of the parent material, a mixture of anionic polymers of maleic anhydride and divinyl-ether of widely varying MWs known as pyran copolymer or Divema (1). The average MW of pyran copolymer was 30,000 but ranged from 18,000 to 32,000 in different batches. The spectrum of MW range was at least from 5500 to 100,000.

Pyran copolymer is an active immunotherapeutic compound which induces interferon (2), activates macrophages (3), activates the reticuloendothelial system (4), and increases resistance to a variety of microbial and viral infections (5). Pyran copolymer has antitumor activity against Lewis lung carcinoma (6), Madison lung carcinoma (7), B16 melanoma (8), various carcinogen-induced tumors (9), L1210 leukemia (10), Rauscher leukemia (11), and mouse mammary carcinoma (12). Regelson and co-workers (13) did a phase I study of pyran copolymer in man. Severe toxic effects were noted which included fever, chills, neurologic symptoms, and bleeding diathesis. One 30-year-old patient had a partial remission. The various MVE compounds have similar biologic properties to a greater or lesser degree, depending on the MW. These include interferon induction, macrophage activation, reticuloendothelial system activation, increased resistance to infection, and antitumor activity (1). Because it has most of the desired biologic activities of pyran copolymer at nontoxic doses, MVE-2 has been selected for clinical trial (14). Preclinical toxicology studies at Adria Laboratories (J. K. Luce, Adria Laboratories, personal communication) showed that the compound MVE-2 was well-tolerated. The only

[1]Received June 24, 1982; revised Dec 7, 1982; accepted Jan 6, 1983.

[2]Supported by Public Health Service grant CA-29769 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Adria Laboratories.

[3]Department of Clinical Immunology and Biological Therapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

†Deceased.

**Reprint requests to:* Adan Rios, MD, Department of Clinical Immunology and Biological Therapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Median hematologic, hepatic, and renal function values before and after treatment with MVE-2

Parameter	Before treatment	After treatment
Hgb (g/100 ml)	11.8 (5.6–17.7)	10.2 (6.0–14.8)
Hematocrit (%)	33.9 (16.1–48.8)	32.9 (18.4–52.9)
Absolute granulocyte count ($\times 10^3$ cells/mm^3)	2844 (225–13,689)	369 (29–13,720)
Platelet count ($\times 10^3$ cells/mm^3)	251 (5–631)	236 (2–510)
Calcium (mg/100 ml)	9.3 (7.6–11.8)	8.9 (7.5–11.9)
BUN (mg/100 ml)	14 (6–34)	16 (5–72)
Creatinine (mg/100 ml)	1.0 (0.5–2.0)	1.0 (0.5–2.8)
Bilirubin (mg/100 ml)	0.5 (0.2–4.1)	0.5 (0.2–5.5)
Alkaline phosphatase (units/dl)	92 (50–350)	96 (51–350)
LDH (units/dl)	212 (99–600)	227 (116–600)
SGOT (units/dl)	28 (12–300)	31 (13–300)

major toxic effect (in dogs) was proteinuria, which was related to both glomerular and tubular damage. The results of a phase I study in cancer patients treated at our institution with MVE-2 are the subject of this report. The drug was well-tolerated at doses up to 1500 mg/m^2 except for reversible proteinuria. Major biologic response modification was not seen.

MATERIALS AND METHODS

Sixty-nine patients with histologically proven disseminated malignant disease were included in the study. At the time of this report 57 patients were evaluable, nine were considered too early, and three were considered inevaluable. Patients were required to have had all previous chemotherapy, radiotherapy, or immunotherapy discontinued at least 2 weeks prior to the study. An informed consent was obtained from all patients according to institutional policy.

Based on animal toxicology data on bolus injection (Adria Laboratories, unpublished observations), an acute LD50 for MVE-2 in man was estimated at 308 mg/m^2 of body surface area. However, using the much more toxic crude pyran copolymer, Regelson and co-workers (13) gave 12 mg/kg or 444 mg/m^2 to advanced cancer patients with serious toxicity but certainly less than that which would be anticipated with a dose comparable to the LD50. Therefore, a starting dose of 25 mg/m^2 of MVE-2 was estimated to be a safe starting dose for the clinical trial.

MVE-2 was supplied in 20-ml vials containing 100 mg as a water-soluble white crystalline solid. MVE-2 was reconstituted with 10 ml of sterile water and was further diluted in 250 ml of 5% dextrose in water to be given iv over 60 minutes. Shorter infusions were not given. The initial ten patients received their infusions over 120 minutes.

Three schedules of administration were used: (*a*) weekly, with doses ranging from 25 to 650 mg/m^2 (32 patients); (*b*) every other week, with doses ranging from 500 to 1200 mg/m^2 (19 patients); and (*c*) every 3 weeks, with doses ranging from 1200 to 1500 mg/m^2 (six patients). The median number of doses was three per patient (range, one to ten). In general, patients were maintained on their starting dose. Dose escalation was not conducted in individual patients. The progressive lengthening of the treatment interval was done in an attempt to overcome the proteinuria problem.

A history was recorded and physical examination was performed prior to treatment. A cbc with differential and platelet counts, chemistry screen, and urinalysis were repeated weekly prior to each course of therapy. Appropriate radiologic examinations and radioscanning were performed as clinically indicated. For immunologic evaluation all patients had measurement of delayed hypersensitivity skin test reactions to a battery of recall antigens consisting of dermatophytin, candida, streptokinase-streptodornase (Varidase), mumps, and purified protein derivative prior to initiation of therapy (15). Patients had serial measurement of natural killer cells (16), antibody-dependent cellular cytotoxicity (17), adherent monocytes in the peripheral blood with the capacity to transform to macrophages,[4] serum lysozyme (18), and serum interferon levels (19). The detailed results of these studies will be part of a separate communication.

Therapy with MVE-2 was discontinued when there was evidence of tumor progression following one or more courses of treatment. Patients were considered evaluable for toxicity if they received at least one course of therapy with 3 weeks of subsequent observation. A partial response was defined as a $\geq$ 50% decrease in the sum of the products of the diameters of all measured lesions lasting for at least 4 weeks. Stable disease was

[4]Hersh EM, Gschwind C, Morris DL, et al. Deficient adherent monocytes in the peripheral blood of cancer patients. Submitted to *Cancer Immunology and Immunotherapy*, 1982.

TABLE 2.—Renal parameters before, during, and after the occurrence of proteinuria (> 1 g/24 hrs) in 7 cancer patients treated with MVE-2

Patient No.	Dose (mg/m²)	BUN (mg/100 ml)			Creatinine (mg/100 ml)			Total protein/albumin (g/100 ml)		
		Before	During	After	Before	During	After	Before	During	After
1	500	8	12	15	1.1	0.8	1.2	6.1/4.1	7.6/4.2	7.0/4.7
2	650	16	16	15	1.0	1.0	1.0	6.7/3.9	6.2/3.7	7/4.8
3	650	15	23	16	0.9	1.3	1.1	/4.2	6.0/4.0	6.1/4.1
4	650	13	12	9	0.9	0.8	1.0	6.9/4.3	6.0/3.5	6.3/3.8
5	650	29	21	21	1.2	1.2	1.4	6.8/4.0	6.9/4.0	7.4/4.0
6	1000	16	17	14	1.0	0.9	1.0	6.5/3.9	6.3/3.8	6.4/3.8
7	1200	9	4	7	0.8	0.8	0.8	7.1/4.1	6.3/3.8	6.2/3.6

defined as a response which was less than a partial response or no further increase of a previously rapidly growing tumor lasting for at least 4 weeks. Progressive disease was defined as an increase by at least 25% in the size of any measured lesion or the development of any new lesions.

RESULTS

Among the patients there were nine with breast cancer, five with colon cancer, nine with lung cancer, ten with melanoma, eight with sarcoma, six with leukemia, five with lymphoma, and five with solid tumors. All patients had advanced metastatic disease. There were 30 males and 27 females, with a median age of 59 years (range, 19–75). They were well-distributed among different cancers. All 57 patients were evaluable for acute toxic effects. None of the patients experienced nausea or vomiting, fever, or any other clinical manifestation of acute toxicity. The drug was tolerated without acute symptomatic side effects at all doses. No significant changes were noted in the parameters of hematologic, hepatic, and renal functions before and after treatment with MVE-2 at all levels of treatment (table 1). However, 25 episodes of proteinuria were documented among the 26 patients treated with $\geq$ 500 mg/m² of MVE-2. Indeed, most if not all patients who received four or more weekly 1-hour infusions of MVE-2 at doses of $\geq$ 500 mg/m² developed reversible proteinuria without changes in BUN, creatinine, or creatinine clearance. This MVE-2-induced proteinuria was asymptomatic and reversible, with a duration of 4–6 weeks. It usually appeared after the second or third dose of MVE-2 and was not accompanied by significant changes in the serum BUN or serum creatinine.

Table 2 summarizes the results in seven typical patients who developed proteinuria in excess of 1 g/24 hours. The maximum amount excreted in a single episode was 4000 mg/24 hours by Patient 4. Table 3 summarizes the total experience with proteinuria in the patients in whom it was observed. The timing of onset and of maximum proteinuria and the median duration of proteinuria are documented. In general, in patients in whom it could be followed, proteinuria resolved within 2 months.

No complete or partial remissions were observed in any of the 57 evaluable patients. However, five patients showed measurable tumor regression while receiving MVE-2. The diagnoses and nature of the responses are detailed in table 4. Patient 1 had nodular poorly differentiated lymphocytic lymphoma; regression of axillary lymphadenopathy was noted after the administration of three doses of MVE-2. Patient 2 had malignant melanoma primary in the scalp with multiple subcutaneous nodules in the scalp and trunk; after four doses of MVE-2, complete regression of one scalp nodule and partial regression of two trunk nodules were noted. Patient 3 had malignant melanoma with the primary located in

TABLE 3.—MVE-2-induced proteinuria in cancer patients*

Dose (mg/m²)	No. of episodes/ No. of patients	Wk of proteinuria		Duration of proteinuria (wks)
		Onset	Maximum	
0–500	1/30	4	7	11
650	15/12	4	7	6
800	1/4	2	5	Not available
1000	3/4	4	6	4
1200	4/4	2	3	3
1500	1/1	5	10	7

*Data shown are median values.

TABLE 4.—Tumor responses* in patients treated with MVE-2

Patient No.	Dose (mg/m^2)	Diagnosis	Response
1	200, every wk	Non-Hodgkin's lymphoma	Regression of lymph nodes
2	300, every wk	Malignant melanoma	Regression of skin nodules
3	650, every other wk	Malignant melanoma	Regression of skin nodules
4	650, every other wk	Leiomyosarcoma	Regression of skin nodules
5	650, every other wk	Breast cancer	Regression of skin nodules

*No patient achieved a partial or a complete response.

the right heel and had developed lesions around the scar of the primary excision at the time of initiation of her treatment with MVE-2; after four doses complete regression of two of the lesions was noted. Patient 4 had metastatic leiomyosarcoma with bone involvement and subcutaneous nodules located in the forehead and over the superior lip; complete disappearance of the nodules in the forehead and the lip was noted after three courses of MVE-2. Patient 5 had breast cancer with multiple nodes located over the left chest (local recurrence); several of these nodules were noted to regress after four courses of MVE-2.

No major effects on host defense parameters were observed. There was no major change in the mean values of each parameter for the total group during therapy. Interferon was not induced (data not shown).

DISCUSSION

MVE-2 is one of the agents identified as a biologic response modifier. These are defined as agents that can modify the structure or function of normal host cells and/or tumor cells so that the outcome of host-tumor interaction favors the host (20). These agents may operate through immunologic and/or nonimmunologic mechanisms. Biologic response modifiers include macrophage activators and interferon inducers (so-called augmenting agents), which is the class to which MVE-2 belongs. The antitumor and macrophage-activating activities of the pyran copolymer derivatives are closely related (1).

This study revealed that at the doses and schedules used, MVE-2 was well-tolerated overall. None of the patients treated experienced fever, chills, nausea and/or vomiting, headaches or myalgias, or physiologic disturbances such as hypertension or hypotension immediately and during the 48 hours after administration of the drug. This is in sharp contrast to the severe symptomatic toxicity of crude pyran copolymer (13). Of note was the lack of hematologic and hepatic toxicity and the absence of significant changes in renal function as determined by BUN and serum creatinine. The only significant side effect encountered was the appearance of asymptomatic proteinuria. The proteinuria was not associated with change in creatinine clearance. Although the proteinuria appeared only in those patients treated with $\geq$ 500-mg/m^2 doses, a strict dose-response relationship was not demonstrated since there were patients receiving higher doses of MVE-2 in whom this proteinuria did not occur. Concurrent serum electrophoresis and urine electrophoresis demonstrated that 80% of the protein excreted was albumin and that there were no significant changes in the serum albumin concentration associated with the proteinuria. Despite these mild findings we concluded that proteinuria may be the dose-limiting toxicity for this agent. This is because similar proteinuria in the dog is associated with morphologic evidence of tubular and glomerular damage. This may preclude achieving a biologically active dose in man or administering the therapy for a long enough period for therapeutic activity. Unpublished observations from Adria Laboratories suggest that more rapid infusion reduces the proteinuria or delays its appearance in dogs. This will be studied in future trials.

No definite conclusions can be made regarding the antitumor activity of MVE-2, although there was some evidence in five patients. There was also little evidence for major immunologic activity in patients as has been seen in the animal models (1). The major question raised by this paper relates to possible future studies of MVE-2. The lack of major biologic response modification is a negative factor while the minor but real tumor regression is a positive one. Proteinuria is not serious or life-threatening but it limits the dose and the duration of treatment. Our immediate plans include: (*a*) study of a more rapid infusion rate to determine if this can overcome the nephrotoxicity, (*b*) study of the pharmacology using ^{14}C-MVE-2, and (*c*) evaluation of other host defense factors which may be modified by MVE-2 such as suppressor cell activity and macrophage tumoricidal activity. Only after these additional studies are completed can further plans for this group of agents be formulated.

REFERENCES

1. Breslow DS, Edward EI, and Newburg NR. Divinyl ether-maleic anhydride (pyran) copolymer used to demonstrate the effect of molecular weight on biological activity. Nature 246:160–161, 1973.
2. Merigan TC. Induction of circulating interferon by synthetic anionic polymers of known composition. Nature 214:416–417, 1967.
3. Braun W, Regelson W, Yajima Y, et al. Stimulation of antibody formation by pyran copolymer. Proc Soc Exp Biol Med 133:171–175, 1970.
4. Munson AE, Regelson W, Lawrence W, et al. Biphasic response of the reticuloendothelial system (RES) induced by pyran copolymer. J Reticuloendothel Soc 7:375–385, 1970.
5. Remington JS, and Merigan TC. Synthetic polyanions protect mice against intracellular bacterial infection. Nature 226:361–363, 1970.
6. Karrer K, Humphreys SR, and Goldin A. An experimental model for studying factors which influence metastases of malignant tumors. Int J Cancer 2:213–223, 1967.
7. Schultz RM, Papamatheakis JD, Luetzeler J, et al. Macrophage involvement in the protective effect of pyran copolymer against the Madison lung carcinoma (M109). Cancer Res 37:358–364, 1977.
8. Morahan PS, and Kaplan AM. Macrophage activation and antitumor activity of biologic and synthetic agents. Int J Cancer 17:82–98, 1976.
9. Morahan PS, Munson JA, Baird LG, et al. Antitumor action of pyran copolymer and tilorone against Lewis lung carcinoma and B-16 melanoma. Cancer Res 34:506–511, 1974.
10. Mohr SJ, Chirigos MA, Smith GT, et al. Specific potentiation of L1210 vaccine by pyran copolymer. Cancer Res 36:2035–2039, 1976.
11. Chirigos MA, Turner W, Pearson J, et al. Effective antiviral therapy of two murine leukemias with an interferon-inducing synthetic carboxylate copolymer. Int J Cancer 4:267–278, 1969.
12. Sandberg J, and Goldin A. Use of first-generation transplants of a slow growing solid tumor for the evaluation of new cancer chemotherapeutic agents. Cancer Chemother Rep 55:233–238, 1971.
13. Regelson W, Shnider BI, Colsky J, et al. Clinical study of the synthetic polyanion pyran copolymer (NSC 46015, Divema) and its role in future clinical trials. *In* Immune Modulation and Control of Neoplasia by Adjuvant Therapy—Progress in Cancer Research and Therapy (Chirigos MA, ed). New York, Raven Press, 1978, vol 7.
14. Breslow DS. Biologically active synthetic polymers. Pure and Appl Chem 46:103–113, 1976.
15. Rasmussen AL, Gutterman JU, Hersh EM, et al. BCG immunotherapy for recurrent malignant melanoma. A study of delayed hypersensitivity to recall antigens and relationship to prognosis. Cancer Immunol Immunother 10:17–26, 1980.
16. Hersh EM, Murphy SG, Quesada JR, et al. Effect of immunotherapy with Corynebacterium parvum and methanol extraction residue of BCG administered intravenously on host defense function in cancer patients. JNCI 66:993–1002, 1981.
17. Poplack DG, Bonnard GD, Holiman BJ, et al. Monocyte-mediated antibody dependent cellular cytotoxicity: a clinical test of monocyte function. Blood 48:809–816, 1976.
18. Kokoslin PL, and DiLuzio NR. Serum lysozyme: an index of macrophage function. J Res Soc 25:85–99, 1979.
19. Rubinstein S, Familletti PC, and Pestka S. Convenient assay for interferons. J Virol 37:755–758, 1981.
20. Mastrangelo M, Hersh EM, and Chirigos MA. Augmenting agents in cancer therapy. Workshop summation. *In* Augmenting Agents in Cancer Therapy (Hersh EM, Mastrangelo M, and Chirigos M, eds). New York, Raven Press, 1980, pp 553–564.

Pharmacokinetics of Therapeutic Doses of Isotopically Labeled Platinum Antitumor Agents in the Mouse and Rat[1,2]

A. B. Robins* and M. O. Leach[3,4]

The pharmacokinetics of the antitumor agents ethylenediamine platinum dichloride and cisplatin have been investigated in mice bearing the ADJ/PC6 plasma cell tumor after therapeutic doses of these agents, labeled either with ^{191}Pt or in the ligand with ^{14}C. The distribution of the radioactive labels has been estimated in tumor, kidney, liver, and intestine in treated animals from 1 hour to 15 days after administration. The two labels, on ligand and on Pt, were equivalent only in some cases with respect to the total radioactivity within a tissue. Patterns of isotope distribution varied with time and tissue and suggested that the metal-ligand bond was dissociated as early as 1 hour after administration. [Cancer Treat Rep 67:245–252, 1983]

To estimate the levels of Pt antitumor agents during pharmacologic investigations, it is usual to employ atomic absorption spectroscopy (1). However, various radioactive isotopes, such as ^{191}Pt (2), ^{193m}Pt (3–5), and ^{195m}Pt (4,6–8), have also been used, and this has led to greater sensitivity in estimation. These isotopes, however, are not readily available and have the drawback of short half-life, which limits their value. Other methods of labeling the Pt complexes have been investigated, such as the use of a radioactive ligand, eg, ^{14}C-ethylenediamine (9,10) and ^{3}H-pyridine (11). This method is potentially useful in a number of "second-generation" Pt compounds, eg, diaminocyclohexane Pt II and homologs of dicyclopentylamine Pt II.

Such a use of radioactive ligands that follows the metabolism of Pt complexes is, however, somewhat open to question, since the metal-ligand bond may be dissociated under some circumstances. It was earlier shown that reactions between ^{14}C-ethylenediamine Pt dichloride ($Pt^{14}C\text{-}enCl_2$) and nucleic acid constituents gave products identical to those from $^{195m}PtenCl_2$ (9). Moreover, the metabolism of $Pt^{14}C\text{-}enCl_2$ and $^{195m}PtenCl_2$ was found to be similar in the rat in vivo (10). Thus, it appeared to be established that a labeled ligand is a valid tracer for Pt, at least in $PtenCl_2$.

Although $PtenCl_2$ is not a clinically useful compound in view of low antitumor activity (*see* below), it is the simplest analog of cisplatin that possesses an easily labeled ligand. It has also been suggested as the basis for future second-generation compounds (eg, ethylenediamine Pt malonate).

The results described here derive from an attempt to follow the pharmacokinetics of Pt antitumor agents within a sensitive tumor line and in organs in the mouse after administration of a therapeutic dose of $PtenCl_2$ labeled either with ^{14}C in the ligand, or with ^{191}Pt. Further investigations have also been made with the rat. It has been shown that the use of a labeled ligand is of limited usefulness. Although the ligand-metal bond remains intact in vitro, under certain physiologic conditions there is evidence that the bond is labile; these conditions will be described.

MATERIALS AND METHODS

Animals

Experiments were conducted on BALB/c mice (25 g) bearing plasmacytoma ADJ/PC6 implanted sc. This tumor exhibited therapeutic indices (LD50/ID90) of 8.1

[1]Received June 14, 1982; revised Dec 17, 1982; accepted Jan 6, 1983.
[2]Supported in part by Johnson-Matthey Research Centre, Sonning Common, Berkshire, England.
[3]Physics Department, Institute of Cancer Research and Royal Marsden Hospital, Sutton, Surrey, England.
[4]We acknowledge the helpful comments, suggestions, and criticisms from J. Drobnik and D. M. Taylor.
*Reprint requests to: Dr. A. B. Robins, Physics Department, Institute of Cancer Research and Royal Marsden Hospital, Downs Rd, Sutton, Surrey SM2 5PX, England.

and 2.2 for cisplatin and $PtenCl_2$, respectively (12). The rats were August strain weighing about 200 g, as used previously (10).

Pt Complexes

$Pt^{14}C\text{-}enCl_2$ was prepared from K_2PtCl_4 and was uniformly labeled ^{14}C-ethylenediamine dichloride (The Radiochemical Centre, Amersham, United Kingdom) by reaction of stoichiometric quantities of ligand and chloroplatinite in the presence of sufficient KOH to neutralize the excess HCl (9). The complex, $PtenCl_2$, separated on warming slightly and was crystalized from 0.1 M HCl. The specific activity of the ligand was 25 μCi/μM, ie, 20% theoretic maximum for two carbon atoms.

The complexes containing ^{191}Pt were prepared from carrier-free $H_2{}^{191}PtCl_6$, supplied by Dr. H. Sharma, Department of Medical Biophysics, University of Manchester, United Kingdom. The Pt isotope will be referred to as ^{191}Pt, since this was the major γ-emitting nuclide initially. However, other nuclides also contribute to the total radioactivity; this will be further discussed in the Appendix.

Unlabeled chloroplatinic acid (50 μmols) was added to the $H_2{}^{191}PtCl_6$ to yield a specific activity of 70–100 μCi/μmol and careful reduction to chloroplatinite carried out by the addition of hydrazinium hydrochloride (13). The resultant $K_2{}^{191}PtCl_4$ was divided into two aliquots. Ethylenediaminedichloride was added to the first aliquot as already described, and $^{191}PtenCl_2$ was isolated and purified. The second aliquot of $K_2{}^{191}PtCl_4$ was subjected to the procedure outlined by Wolf et al (14). The K_2PtCl_4 was reacted with KI and ammonia to yield $Pt(NH_3)_2I_2$, which was isolated and dried. $AgNO_3$ was added to this solid in solution, which yielded a precipitate of AgI and to $Pt(NH_3)_2(NO_3)_2$ in solution. All reagents were used in stoichiometric amounts. The addition of a slight excess of KCl yielded $cisPt(NH_3)_2Cl_2$ (cisplatin), which was crystalized from 0.1 M HCl.

The Pt complexes were assayed for purity by chromatography on PEI cellulose plates, eluting with 0.1 M $NaClO_4$. Unchanged chloroplatinite remained at the origin, and unchanged ethylenediamine moved with the solvent front. The R_f was 0.62 for $PtenCl_2$ and 0.55 for cisplatin. Both complexes were > 95% pure by this separation.

Administration

To mice.—The Pt complexes were separately dissolved in isotonic saline to a concentration of 1 mg/ml. Fresh solutions were injected ip: $PtenCl_2$ (10 mg/kg) and cisplatin (2 mg/kg). All animals were used in groups of three for each data point.

To rats.—$PtenCl_2$ (3 mg/kg) and cisplatin (1 mg/kg) were injected. Again, groups of three animals were used.

Biochemical Analysis

Groups of animals were killed at the appropriate times, and tumor, kidney, liver, and portions of intestine were excised and weighed. The biochemical fractionation procedure has been outlined elsewhere (15). Tissues were homogenized in 5-ml aliquots of 0.2 M ice-cold perchloric acid ($HClO_4$), followed by two rinses of the homogenizer with 2.5 ml of 0.2 M $HClO_4$. After centrifugation and separation of the supernatant, the precipitate was washed twice more with 2.5-ml aliquots of 0.2 M $HClO_4$, and supernatants were pooled to give the "acid-soluble fraction" (15–16 ml). The addition of 5 ml of 0.3 M KOH to the precipitate and incubation overnight at 37°C led to hydrolysis of the RNA. The addition of 3.2 ml of 1 M $HClO_4$ neutralized the KOH and produced a 0.2 M suspension with respect to $HClO_4$, which was cooled to 4°C. Centrifugation was carried out, followed by two washings of the precipitate with 2.5 ml of 0.2 M $HClO_4$, and the pooled supernatant solutions were the "RNA fraction" (13 ml). The precipitate was extracted with 2.5 ml of 1 M $HClO_4$ at 68°C for 30 minutes followed by cooling and centrifugation. This labilization of DNA was repeated another three times for 30 minutes each. The pooled supernatants were the "DNA fraction" (10 ml). The residual precipitate was homogenized in 5 ml of 0.3 M KOH to form the "protein fraction." The "total radioactivity" is the arithmetic sum of the four fractions; in ^{191}Pt, this was within ± 10% of the total tissue radioactivity, measured immediately after excision. The distribution of the radioactivity between different biochemical fractions will be reported in a separate communication.

Estimation of Radioactivity

Radioactivity from ^{191}Pt was measured in an Intertechnique scintillation counter (CG 30), with an efficiency of 37%.

The β-activity was measured in an Intertechnique liquid scintillation counter (SL40) using a detergent scintillation mixture (Cocktail T, Hopkin, and Williams, United Kingdom). By choosing appropriate standards and by measuring radioactivity in both β and γ counters, ^{14}C activity could be obtained by subtraction, with an efficiency of 85%, when both ^{191}Pt and ^{14}C were present. A more detailed outline of the factors that may affect the observed count rate of ^{191}Pt is given in the Appendix.

Radioactive samples were all compared with an aliquot of the solution for injection. It was found that the observed half-life of ^{191}Pt was initially about 4 days.

The data in figures 1–5 are expressed in terms of the mean and standard error of the mean for a minimum of

three animals at each data point. Each graph is a composite of pooled data from several experiments.

RESULTS

In figure 1 the ratio of the total radioactivity of ^{191}Pt to the total radioactivity of ^{14}C is expressed as a function of time for the mouse up to 8 hours after administration. In all the ratios described here, the values are derived from the percent injected dose per gram of wet tissue. In the mouse, the tissues are liver, kidney, tumor, and intestine. It is evident that between 1 and 8 hours, values of the ratio remain fairly constant, although the different tissues show deviations from unity. The dashed line represents the ratio for radioactivity levels for serum in the same animals.

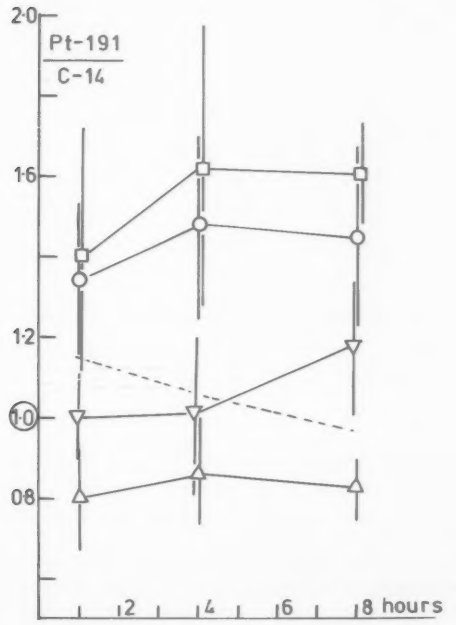


FIGURE 1.—Ratio of total ^{191}Pt to total ^{14}C in tissues of the mouse. -o- = kidney; -Δ- = liver; -∇- = intestine; -□- = tumor; --- = plasma. Data are from 1 to 8 hrs. Errors are SEM from 3 animals at each data point.

Figure 2 illustrates the comparable situation in rat tissues, for liver, kidney and intestine only. A comparison between figures 1 and 2 shows that although both mouse and rat results are almost identical with respect to the liver values, there is a considerable difference between the species when the kidney and intestine values are compared. This is demonstrated further when the values at longer time intervals are compared, eg,

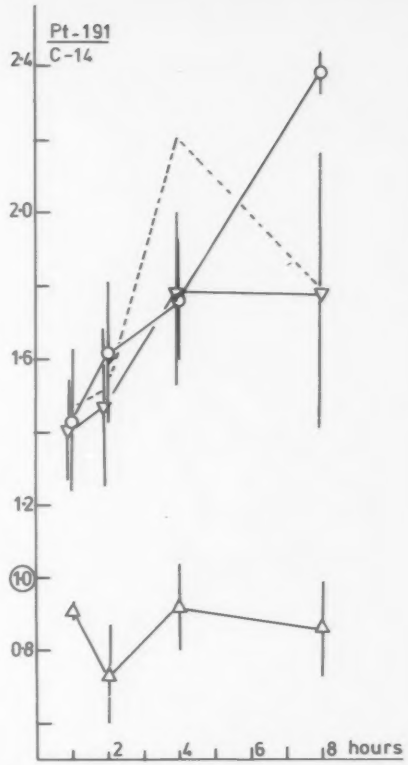


FIGURE 2.—Ratio of total ^{191}Pt to total ^{14}C in tissues of the rat. -o- = kidney; -Δ- = liver; -∇- = intestine; --- = plasma. Data are from 1 to 8 hrs.

figures 3 and 4. In figure 4, the rise in the ratio for the rat kidney is dramatic; this is foreshadowed in figure 2. A similar rise is seen in values for the mouse kidney but at longer time intervals (fig 3), which had not been apparent in the first few hours (fig 1).

Figure 5 shows plotted ratios between the total radioactivity levels of ^{191}Pt in the same tissues when the ^{191}Pt had been administered either as cisplatin or as $PtenCl_2$. Although calculated at longer time intervals, the ratio varies only between 1 and 1.5 or 2. In the first few hours there is considerable divergence and in particular a difference between the mouse and the rat.

DISCUSSION

As stated previously, the use of a radioactive label on the ligand attached to the Pt atom could be an alternative method by which to follow the pharmacokinetics of the Pt antitumor agents. Such ligands are tightly bound to the metal atom and are resistant to chemical

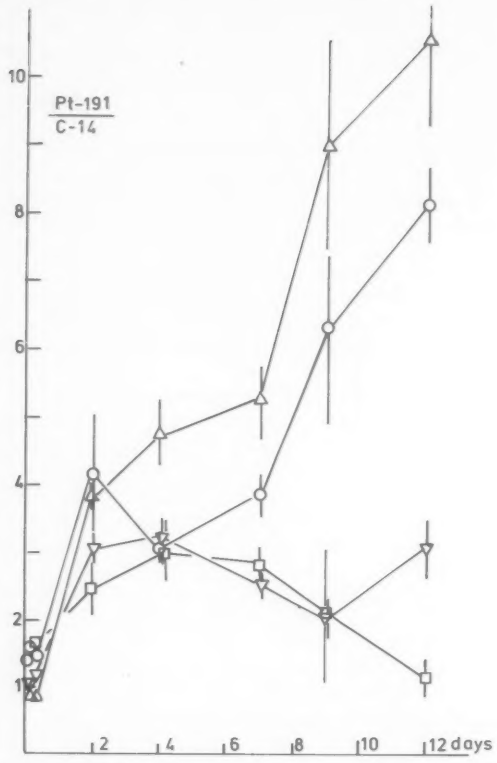


FIGURE 3.—Ratio of total ^{191}Pt to total ^{14}C in mouse tissue. Symbols are the same as for fig 1. Data are from 1 hr to 12 days. (Errors are not given for early time points to avoid confusion.)

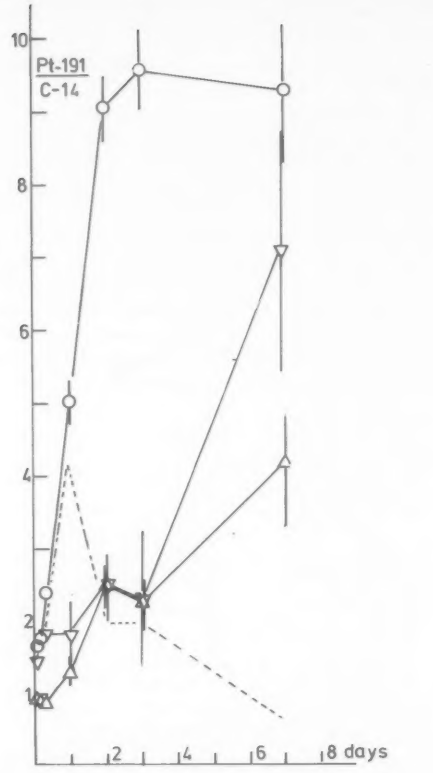


FIGURE 4.—Ratio of total ^{191}Pt to total ^{14}C in rat tissue. Symbols are the same as for fig 2. Data are from 1 hr to 7 days.

treatment and reaction with nucleophiles such as nucleic acid constituents (9). However, it is obvious from the results described here that this is not the case in vivo, except under certain circumstances; eg, in the intestine of the mouse for up to 8 hours after administration (fig 1). Other tissues of the same animal at the same time intervals show variations suggesting that there has been dissociation of the ligand. In the liver of both mouse and rat the ratio of radioactive labels suggests that ^{14}C is being more strongly retained than ^{191}Pt, and the reverse is true in kidney and tumor (figs 1 and 2). It is significant that such differences between tissues are already well-established as early as 1 hour after administration. The plasma levels of both isotopes appear to be in a constant ratio throughout the first few hours in the mouse, which suggests, but does not prove, that the circulating Pt complex may be undissociated. Values > 1 for $^{191}Pt/^{14}C$ indicate a preferential binding of Pt, while ^{14}C is lost from the tissue.

In the rat, even at short time intervals, evidence for dissociation is most pronounced in kidney and intestine (fig 2). Moreover, in this species the plasma isotope ratio is considerably different from unity and demonstrates a rapidly changing picture.

At longer time intervals the divergences already described are greatly enhanced and appear in the mouse also (fig 3). At periods > 4 days, the tissue patterns appear to group into pairs with kidney and liver following an upward trend as Pt is retained and the ligand is lost. The other pair of tissues, tumor and intestine, follow a different pattern at ≥ 4 days, when the isotope ratio decreases again toward unity. The individual values from which ratios were calculated demonstrate that the level of ^{14}C in the tissue fell less rapidly at ≥ 4 days. At the present time, no investigation has been carried out as to the nature of the ^{14}C-labeled material, which may be intact ethylenediamine or a metabolite produced from it.

Because divergences begin to appear a very short time after administration of the compound, an enzy-

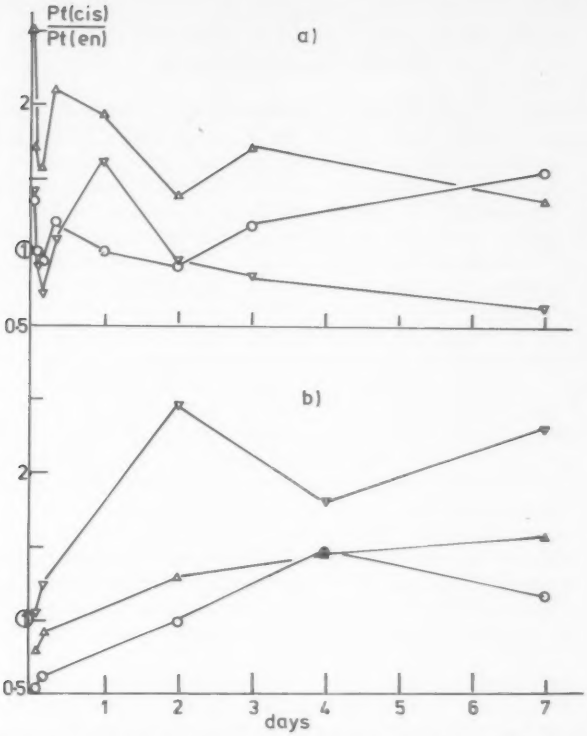


FIGURE 5.—Ratio of total ^{191}Pt from cisplatin to total ^{191}Pt from $PtenCl_2$. a) = rat and b) = mouse. -o- = kidney; -Δ- = liver; -∇- = intestine.

matic process may be an explanation. However, this remains to be demonstrated. Another very likely possibility is the rapid binding of the Pt complex at a site where sulphur is present, followed by translabilization of the ligand (16). There is accumulating evidence for the involvement of Pt-S bonds in vivo, and sulphur-containing compounds have been successfully employed to reverse Pt nephrotoxicity (17,18) and DNA reactions in vitro (19).

More investigation is needed into the pharmacokinetics of the Pt antitumor agents. These differ from one another in their selectivity and toxicity, although our data suggest that ligand dissociation has occurred quite quickly within the tissues. The situation with ligands other than ethylenediamine may well be different, and second-generation compounds will have to be individually examined from the point of view of possible metal-ligand dissociation. It is possible that the active Pt species is the same in all cases and that the pharmacologic differences are mediated by the ligands and by the leaving groups; this is still a matter for speculation.

The distribution of ^{191}Pt is comparable in mice and rats from 1 or 2 days after administration, whether the isotope originated from $PtenCl_2$ or from cisplatin (fig 5). However, at the earliest times after administration the rat shows a pronounced increase of cisplatin vis-a-vis $PtenCl_2$. This has not been further investigated.

It can be concluded from our data that the use of a labeled ligand by which to follow the pharmacokinetics of the platinum moiety is open to question. Direct estimation of Pt or the use of Pt nuclides appears to be essential for studies of the fate of these drugs in vivo. However, none of these methods provides information on the cytotoxicity of the Pt retained in the tissues. The development of a sensitive in vitro method for assessing the toxicity of such retained metal after treatment in vivo is needed to answer this important question.

APPENDIX
Factors Affecting Observed Count Rate From Pt Isotopes Used in These Experiments

When an osmium target is bombarded with alpha particles from a cyclotron, a mixture of Pt isotopes is produced. Many of the isotopes have a relatively short half-life (< 11 hours) and would have therefore decayed to an insignificant level before the experimental work was carried out. Likewise, negligible activity of isotopes with a long half-life would be produced. Of the experimentally useful isotopes produced, ^{191}Pt has a half-life of 3 days and is the major γ-emitting isotope produced, ^{193m}Pt has a similar half-life, and ^{188}Pt is particularly useful since it has a 10.2-day half-life, permitting experiments and sample counting to be continued over a longer period than is possible with the single-isotope sources that are commercially available (^{193m}Pt and ^{195m}Pt).

Many of these Pt isotopes decay to radioactive Ir daughters, and some of these have a half-life of sufficient length that unless they are removed by a chemical separation, they will also contribute to the total sample count rate. The major Ir isotopes present are ^{189}Ir (half-life, 13.1 days) and ^{188}Ir (half-life, 41.5 hours); the latter is usually in equilibrium with its longer-lived parent. A small activity of ^{192}Ir is also present. The precise activities of all of these isotopes at any time will be a function of the irradiation conditions, the chemical processes to which the isotopes have been subjected (including those occurring in the animals used), and the time elapsed since irradiation and any chemical processes that caused the separation of Ir and Pt. The observed count rate for the mixture and the relative importance of the Pt and Ir isotopes will be a function of the detection method (ie, liquid scintillation counter or well counter) and the energy window settings used. These latter factors will vary among laboratories, and we have therefore measured the activities of the relevant isotopes from γ decay lines, a result that is not dependent on detection method.

The major purpose of this appendix is to determine whether there was any preferential removal of Ir from Pt in the different animal tissues considered in the study. To determine this in a relatively simple manner, only three isotopes have been measured: ^{191}Pt, ^{188}Pt, and ^{188}Ir. The equilibration of ^{188}Ir with ^{188}Pt has also been observed, and the results are expressed in terms of the ratio of ^{188}Ir activity to ^{188}Pt activity.

By electron capture, ^{188}Pt decays to a radioactive daughter; ^{188}Ir (half-life, 41.5 hrs) and the proportion of ^{188}Ir to ^{188}Pt would be expected to vary in the course of normal chemical preparations, possibly causing some variations in the observed γ or β count rate, depending upon the degree of equilibration of ^{188}Ir with its parent. To examine whether this ratio varied during the experimental work reported here, a number of samples were analyzed by γ-spectroscopy using a 50-cm^3 GeLi detector. The ratios of ^{188}Ir to ^{191}Pt and ^{191}Pt to ^{188}Pt were also examined as control measurements. The results from these measurements are shown in table 1. The γ energies studied were 155.0 keV for ^{188}Ir, 187.6 and 195.1 keV for ^{188}Pt, and 172.19 and 178.96 keV for ^{191}Pt. The observed activity of each isotope was calculated from the summed counts in the full energy peak, corrected for the abundance, per decay of the parent, for each line. Since the counts from the ^{191}Pt lines were not separated, a correction was made for the 7% of 178.96-keV transitions that would result from a 172.19-keV transition. A correction has also been made for a low abundance ^{191}Pt γ-line at 187.69 keV, which amounted to up to 3% of the disintegration attributed to ^{188}Pt. The nuclear transition data were obtained from Bowman and MacMurdo (20) and Browne (21). No correction has been made for the varying efficiency of the GeLi detector at these different energies or for self-absorption in the samples. The former effect is constant for the ratios quoted but will cause the absolute abundance of Ir to be overestimated by some 20%. The latter effect will be small since all the samples were of similar volume.

Table 1 shows that in measurements made shortly after preparation, eg, Sample 6, the proportion of ^{188}Ir is very low, which would be expected for a freshly prepared Pt compound. Repeated counts of Sample 2 show that by Day 5 the Ir had almost equilibrated with the Pt, which is in accord with the expected buildup. The expected ratio

TABLE 1.—Ratios of ^{188}Ir: ^{188}Pt and ^{191}Pt: ^{188}Pt activities for $PtenCl_2$ and cisplatin before administration, and in some tissue samples

Source of isotope	No. of days elapsed from day of preparation	^{188}Ir:^{188}Pt*	^{191}Pt:^{188}Pt*
(1) Cisplatin (solid)	5	1.27 ± 0.01	3.17 ± 0.07
(2) Cisplatin in saline as injected	5	1.31 ± 0.02	3.5 ± 0.2
(3) Sample 2	6	1.33 ± 0.02	1.7 ± 0.5
(4) Sample 2	7	1.34 ± 0.1	1.85 ± 0.06
(5) Cisplatin in saline	26	1.41 ± 0.01	No observed ^{191}Pt
(6) $PtenCl_2$ as injected	0	0.08 ± 0.02	3.1 ± 0.2
(7) $PtenCl_2$ in mouse blood†	1	0.29 ± 0.04	2.1 ± 0.2
(8) $PtenCl_2$ in mouse tumor†	2	0.64 ± 0.07	2.3 ± 0.4
(9) $PtenCl_2$ in mouse liver†	2	0.59 ± 0.03	2.1 ± 0.2
(10) $PtenCl_2$ in mouse kidney†	2	0.56 ± 0.03	2.3 ± 0.25
(11) $PtenCl_2$ as injected	2	0.59 ± 0.01	1.9 ± 0.06

*Values = mean ± SD.

†Samples from mice, all injected with $PtenCl_2$ and killed after 8 hrs.

for absolute abundances is 0.98. The final maximum calculated equilibration ratio is 1.20; this is achieved after about 16 days and is shown in Sample 5, which has the same composition as Sample 2. Once a Pt isotope is injected into the organism, the metabolic pathways of the Pt compound and of ^{188}Ir may differ, leading to the possibility of regional differences in the Ir:Pt ratio and a reduction in this ratio as a result of preferential removal of ^{188}Ir. The results from Samples 8–10 give no evidence of regional variations and when compared with the noninjected solution, Sample 11, they show no significant indication of preferential removal of ^{188}Ir. However, the time between the killing of the animal and counting of the sample was approximately 40 hours, and the subsequent buildup of Ir would, within experimental error, mask any of these effects. The theoretic ratios of Ir:Pt following decays of 48 and 40 hours are 0.59 and 0.51, respectively. The ratio observed in Sample 7 was rather low, indicating that preferential removal of Ir might have occurred.

The effects of Ir buildup can be minimized either by completing experiments in a very short period following radiopharmaceutic preparations or by delaying counting of samples until several days after removal from the animal. A more detailed discussion of methodology arising from the presence of Ir isotopes has been reported (22).

REFERENCES

1. LEROY AF, WEHLING ML, SPONSELLER HL, ET AL. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184, 1977.
2. RENSHAW E, and THOMSON AJ. Tracer studies to locate the site of platinum ions within filamentous and inhibited cells of *E. coli*. J Bacteriol 94:1915–1918, 1967.
3. DECONTI RC, TOFTNESS BR, LANGE RC, ET AL. Clinical and pharmacological studies with *cis*-diammine dichloroplatinum II. Cancer Res 33:1310–1315, 1973.
4. LANGE RC, SPENCER RP, and HARDER HC. The antitumour agent *cis* Pt $(NH_3)_2Cl_2$: distribution studies and dose calculations for ^{193m}Pt and ^{195m}Pt. J Nucl Med 14:191–195, 1973.
5. TOTH G, and TARJAN GY. Synthesis, quality control and organ specificity of ^{193m}Pt labelled *cis*PtII. *In* Radioactive Isotope in Klinik und Forschung. Egermann, Vienna, 12:223–227, 1976.
6. DOUPLE EB, HOWDEN FM, and HOESCHELE JD. Distribution of platinum chemotherapeutic complexes in normal tissues and in tumours. Int J Radiat Oncol Biol Phys 5:1387–1391, 1979.
7. SMITH PHS, and TAYLOR DM. Distribution and retention of the antitumour agent *cis*^{195m}PtII in man. J Nucl Med 15:349–351, 1974.
8. TAYLOR DM. The pharmacokinetics of *cis*PtII in animals and in man: relation to treatment scheduling. Biochimie 60:949–956, 1978.
9. ROBINS AB. The reaction of ^{14}C labelled platinum ethylenediaminedichloride with nucleic acid constituents. Chem Biol Interact 6:35–45, 1973.
10. TAYLOR DM, JONES JD, and ROBINS AB. Metabolism of platinum ^{14}C-ethylenediaminedichloride in the rat. Biochem Pharmacol 22:833–839, 1973.

11. Howle JA, Gale GR, and Smith AB. A proposed mode of action of antitumour platinum compounds, based upon studies with *cis*-dichloro(G-^{3}H)dipyridine platinum II. Biochem Pharmacol 21:1465–1475, 1972.
12. Cleare MJ, Hydes PC, Malerbi BW, et al. Antitumor platinum complexes: relationships between chemical properties and activity. Biochimie 60:835–850, 1978.
13. Kauffman GB, and Cowan DO. *Cis*- and *trans*-dichlorodiammine platinum II. New York, McGraw-Hill, Inorg Synth 7:236, 1963.
14. Wolf W, Manaka PC, and Ingalls RB. Radiopharmaceuticals in clinical pharmacology: ^{195m}Pt-cisdiamminedichloroplatinum II. *In* Radiopharmaceuticals and Labelled Compounds. Vienna, IAEA-5M-171/51, 2:205–221, 1973.
15. Robins AB, and Taylor DM. ^{123}I-iododeoxyuridine: a potential indicator of tumor response to treatment. Int J Nucl Med Biol 8:53–63, 1981.
16. Howe-Grant ME, and Lippard SJ. Aqueous platinum II chemistry; binding to biological molecules. *In* Metal Ions in Biological Systems. Marcel Dekker, 11:63–125, 1980.
17. Borch RF, and Pleasants ME. Inhibition of cisplatinum nephrotoxicity by diethyldithiocarbamate rescue in a rat model. Proc Natl Acad Sci USA 76:6611–6614, 1979.
18. Howell SB, and Taetle R. Effect of sodium thiosulphate on *cis*-dichlorodiammineplatinum(II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat Rep 64:611–616, 1980.
19. Filipski J, Kohn KW, Prather R, et al. Thiourea reverses cross-links and restores biological activity in DNA treated with *cis* Pt II. Science 204:181–183, 1979.
20. Bowman WW, and MacMurdo KW. Radioactive decay gammas. Atomic Data and Nuclear Data Tables 13:89, 1974.
21. Browne E. Nuclear Data Sheets, A = 191. 30:653, 1980.
22. Leach MO, and Robins AB. Factors affecting the use of Pt-191 as a tracer for platinum anti-tumor agents. *In* Proceedings of the 3rd World Congress of World Federation of Nuclear Medicine and Biology, Paris, Aug 1982.

Effect of Nutritional Status on the Hepatobiliary Excretion of Methotrexate in the Rat[1,2,3]

V. Bruce Grossie, Jr,* and Ti Li Loo[4]

The effect of induced protein-calorie malnutrition (PCM) and its subsequent nutritional repletion with chow or total parenteral alimentation (TPA) on the hepatobiliary excretion of methotrexate (MTX) was determined in the Sprague-Dawley rat. Compared with full-fed controls, PCM resulted in a decrease in body and liver weight, bile flow, and the cumulative excretion of MTX but a consistently higher plasma MTX concentration at each time-point measured. Repletion with chow and TPA resulted in a gain in body weight and an increase in liver weight. Chow repletion resulted in an increase in bile flow and biliary excretion of MTX equal to that for the controls. Repletion with chow also resulted in a consistently lower plasma MTX concentration as compared with PCM rats. Repletion of PCM via TPA did not affect biliary, or plasma, MTX concentrations although the cumulative biliary excretion of MTX was further decreased. The biliary clearance of MTX was decreased by PCM. The clearance for rats repleted with chow, however, was greater than that for the controls. The clearance for PCM rats repleted via TPA was equal to that for PCM rats but less than that for the controls. When expressed as a function of liver weight and compared with PCM rats, the clearance for rats repleted with chow was increased while that for malnourished rats repleted parenterally was decreased. These studies suggest that the biliary excretion of MTX in the rat is a carrier-mediated process that is sensitive to the nutritional status of the host and independent of the effect of nutritional status on liver weight. [Cancer Treat Rep 67:253–257, 1983]

Patients with cancer are often anorectic due to their disease or treatment. This decrease in food intake leads to protein-calorie malnutrition (PCM) which reduces the patient's capacity to withstand therapy and increases the risk of death (1–3). Enteral and parenteral hyperalimentation has been used successfully for nutritionally repleted malnourished cancer patients; they gain weight and are less susceptible to the toxic side effects of therapy (3–5). While similar results have been demonstrated in the rat given total parenteral alimentation (TPA) (5,6), the basic mechanism by which this protection occurs is not understood.

The nutritional status of man and experimental animals has been demonstrated to affect drug pharmacokinetics and response. Malnutrition (7), glucose given orally or parenterally (8–10), and the quantity and quality of dietary lipids (11,12) have been shown to affect the hepatic drug-metabolizing enzyme systems. Malnutrition has been reported to have diverse effects on different organs, which could adversely affect the response to various drugs (13).

We recently demonstrated that the toxicity of methotrexate (MTX) and tissue dihydrofolate reductase content are affected by malnutrition (14). The biliary excretion of MTX is one important excretory route in the rat (15) and possibly in man (16,17). Excretion of the drug by this route may cause local irritation to the gut by direct contact, be metabolized by gut bacteria, undergo enterohepatic circulation, and/or be eliminated in the feces, all of which will affect the dose of drug tolerated by the host. We now present evidence that induced PCM and its subsequent nutritional repletion

[1]Received June 16, 1981; revised Dec 23, 1982; accepted Jan 13, 1983.

[2]Supported by American Cancer Society grant IN-121A, and by contract N01-CM-87185 (Division of Cancer Treatment) and Public Health Service grant CA-11520 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Presented in part at the Annual Meeting of the American Association for Cancer Research, San Diego, CA, May 28–31, 1981.

[4]Department of Developmental Therapeutics, Section of Pharmacology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, TX.

Reprint requests to: V. Bruce Grossie, Jr, PhD, Department of Developmental Therapeutics, Section of Pharmacology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Body weights of malnourished and nutritionally repleted rats

Nutritional status	Body weight (g)*		
	Initial	Malnourished	Repleted
PCM	276 ± 4	196 ± 3	Not applicable
PCM-chow	277 ± 4	194 ± 3	272 ± 4†
PCM-TPA	271 ± 3	196 ± 5	221 ± 3†

*Mean ± SE.
†Differs significantly ($P < 0.05$) from the respective mean prior to repletion.

via TPA or chow affect the hepatobiliary excretion of MTX in the Sprague-Dawley rat.

METHODS

Animal Care and Feeding

Weanling male Sprague-Dawley rats were obtained from Simonsen Laboratories (Gilroy, CA) and were reared three per plastic cage with wood shaving bedding; food and water were administered ad libitum during the growth period. The controls were housed in wire-bottom cages and fed ad libitum. The body weight of the controls after 14 days was equal to that of rats just prior to the induction of PCM. The hepatobiliary excretion of MTX for the controls was determined after 14 days of feeding.

PCM was induced by reducing the daily amount of chow (Purina Rodent Lab Chow-5001) administered to 10 g/rat. On Day 14 of the restricted feeding regimen the rats were divided into three groups. The hepatobiliary excretion of MTX was determined immediately in the PCM group while a second group was returned to the original cage and fed chow at 125%–150% of their normal intake for 7 days (PCM-chow). A third group received total parenteral alimentation (PCM-TPA) for 7 days at the maximum infusion volume.

Those rats to receive TPA were anesthetized with an im treatment of 44 mg/kg of ketamine and 6.1 mg/kg of acepromazine; cannulas were inserted into the superior vena cava via the jugular vein as previously described (18). The iv infusate administered was composed of: FreAmine II (600 ml), 70% dextrose in H_2O (425 ml), calcium gluconate (2.4 g), sodium phosphate (12.1 mEq), magnesium sulfate (3.7 mEq), sodium acetate (16.1 mEq), sodium chloride (10.7 mEq), potassium chloride (27.8 mEq), potassium acetate (37.5 mEq), and multiple vitamins (0.5 ml). The concentration of amino acids was 4.6% at a calorie-to-nitrogen ratio of 135:1 and was similar to the regimen used in other studies on the effects of iv hyperalimentation in rats (6). A Holter roller pump was used to maintain a constant flow of infusate that was formulated in a laminar flow hood and changed every 48 hours. The daily infusion volume was 40 ml for Day 1, 50 ml for Day 2, and 50–55 ml for the next 7 days. The urinary output was monitored throughout the infusions.

Biliary Excretion

After completion of the respective dietary regimen, the rat was anesthetized with 60 mg/kg of ketamine im. The abdomen was clipped free of hair, the rat placed on a surgical board under a heat lamp, and a midline incision made. Supplemental anesthesia with ether was used as needed. The common bile duct was gently isolated and ligated with 4-0 silk next to the duodenum and an Intramedic PE-10 cannula was inserted; bile flow was established immediately. The duct was secured to the cannula with 4-0 silk and the wound closed with autoclips, leaving one end of the cannula exposed. Bile was collected for 10 minutes to allow the flow to sta-

TABLE 2.—Liver weight and bile flow of malnourished and nutritionally repleted rats

Nutritional status*	No. of rats	Liver weights†		Bile flow† (μl/min)
		g	% of body weight	
Control	8	11.7 ± 0.4	4.2 ± 0.1	27.3 ± 2.1
PCM	20	5.5 ± 0.3‡	2.8 ± 0.1‡,§	16.2 ± 0.5‡
PCM-chow	21	9.9 ± 0.3§	3.6 ± 0.1‡,§	25.2 ± 1.0
PCM-TPA	14	7.7 ± 0.2‡,§,¶	3.5 ± 0.1‡,§	17.5 ± 0.6‡,¶

*Control rats were fed ad libitum; final body weight = 282.1 ± 3.4 g.
†Mean ± SE.
‡Significantly different ($P < 0.05$) as compared with control rats.
§Significantly different ($P < 0.05$) as compared with PCM rats.
¶Significantly different ($P < 0.05$) as compared with PCM-chow rats.

bilize. Chromatographically pure 3′,5′,7n(H^3)-methotrexate (19) was injected via the tail vein at 5 mg and 5 μCi/kg of body weight. The bile was collected at various intervals and the radioactivity was determined with a Packard Tricarb Model 2650 liquid scintillation spectrometer. Blood was collected from the tail, the plasma was separated by centrifugation, and the radioactivity was determined. The biliary clearance of MTX was calculated by dividing the total MTX excreted (μg of MTX) during an 80-minute interval by the area under the plasma concentration versus time curve (AUC).

Difference between means was analyzed for statistical significance ($P < 0.05$) using Student's t test. All data are presented as the mean ± SE.

RESULTS

Rats that were malnourished by reducing the chow intake lost 28%–30% of their initial body weight (table 1). This feeding regimen results in a sharp decrease in body weight during the initial 24 hours and a less severe but consistent daily weight loss for the remainder of the 14-day study. Repletion with chow resulted in an almost complete recovery of body weight equal to that at the beginning of the experiment. Repletion with parenteral alimentation resulted in a smaller yet significant increase in body weight as compared with the respective body weights prior to repletion (table 1).

The nutritional status of rats has a significant effect on the liver weight and bile flow (table 2). PCM rats had consistently smaller livers than those rats fed ad libitum, while the repletion regimens resulted in significant yet incomplete recovery of liver weight. As a percent of body weight, the livers for PCM-chow rats were slightly larger as compared with those for PCM-TPA rats although neither of the repletion regimens resulted in a liver-to-body weight ratio equal to that for the control rats. The absolute bile flow was decreased for PCM rats as compared with full-fed controls. Repletion with chow resulted in an increase in the absolute bile flow to a rate equal to the controls, while parenteral repletion had little effect.

TABLE 3.—Effect of nutritional status of rats on cumulative biliary excretion of MTX

Nutritional status*	% of dose†
Control	84.3 ± 2.1
PCM	66.2 ± 1.9‡
PCM-chow	75.3 ± 2.3§
PCM-TPA	56.7 ± 2.7§,¶

*Control rats were fed ad libitum; final body weight = 282.1 ± 3.4 g.
†Cumulative % of the 5-mg/kg dose excreted in the bile after 80 mins.
‡Significantly different ($P < 0.05$) as compared with control rats.
§Significantly different ($P < 0.05$) as compared with PCM rats.
¶Significantly different ($P < 0.05$) as compared with PCM-chow rats.

The cumulative biliary excretion of MTX was reduced when rats were malnourished (table 3). Repletion with chow resulted in an increase in the cumulative biliary excretion of MTX as compared with that for PCM rats but it remained equal to that for the controls. Parenteral repletion resulted in a further decrease in the cumulative excretion as compared to that for PCM rats.

The effect of nutritional status on the biliary MTX concentration is illustrated in table 4. Preliminary results demonstrated a rapid increase in the biliary MTX concentration, with the peak occurring 8–10 minutes after an iv injection of MTX at 5 mg/kg; the concentration declined slowly thereafter. A 10-minute collection time was therefore used for the initial two collection periods followed by 20-minute periods until the end of the study. For all groups, the biliary MTX concentration increased during the two initial 10 minutes of collection periods and then decreased at each successive collection period thereafter up to 80 minutes. The biliary MTX concentration for PCM rats was equal throughout the experiment to that for the controls. For chow-repleted rats, the biliary MTX concentration was higher during the initial collection period as compared to that of PCM rats but lower during the latter collection periods as compared with PCM and control rats. The biliary MTX concentration for TPA-repleted rats was lower than that for the other rats during the initial

TABLE 4.—Effect of nutritional status of rats on biliary MTX concentration*

Nutritional status†	Time (mins)‡				
	0–10	10–20	20–40	40–60	60–80
Control	723 ± 58	955 ± 63	544 ± 46	327 ± 35	206 ± 28
PCM	802 ± 53	922 ± 32	471 ± 25	275 ± 18	193 ± 13
PCM-chow	1098 ± 43§	1137 ± 68§	489 ± 40	233 ± 23§,¶	134 ± 15§,¶
PCM-TPA	604 ± 47§,¶,**	879 ± 59**	464 ± 43	259 ± 29	185 ± 24

*Values = mean ± SE (μg/ml).
†Control rats were fed ad libitum; final body weight = 282.1 ± 3.4 g.
‡Time interval after iv treatment with 5 mg/kg of MTX.
§Significantly different ($P < 0.05$) as compared with PCM rats.
¶Significantly different ($P < 0.05$) as compared with control rats.
**Significantly different ($P < 0.05$) as compared with PCM-chow rats.

TABLE 5.—Effect of nutritional status on plasma MTX concentration*

Nutritional status†	Time (mins)‡			
	5	20	50	80
Control	15.4 ± 1.4	6.2 ± 0.4	1.9 ± 0.2	1.3 ± 0.3
PCM	19.2 ± 1.4§	7.6 ± 0.6	3.6 ± 0.3§	2.4 ± 0.3§
PCM-chow	12.3 ± 1.4¶	3.8 ± 0.4§,¶	1.2 ± 0.2§,¶	0.7 ± 0.2§,¶
PCM-TPA	17.5 ± 2.4	7.4 ± 1.4	4.3 ± 1.2§,**	2.4 ± 0.7§,**

*Values = mean ± SE (μg/ml).
†Control rats were fed ad libitum; final body weight = 282.1 ± 3.4 g.
‡Time after iv treatment with 5 mg/kg of MTX.
§Significantly different ($P < 0.05$) as compared with control rats.
¶Significantly different ($P < 0.05$) as compared with PCM rats.
**Significantly different ($P < 0.05$) as compared with PCM-chow rats.

10-minute collection period but equal during the remaining time periods.

The MTX concentration in plasma for PCM rats was consistently higher than in the plasma of the control group at each of the respective time points (table 5). Repletion with chow resulted in a decrease in the plasma concentration of MTX as compared with PCM rats at 5, 20, 50, and 80 minutes after iv administration, while the plasma concentration of MTX for PCM-TPA rats was equal to that for PCM rats at these times.

The effect of malnutrition and nutritional repletion on the biliary clearance of MTX is shown in table 6. The clearance for malnourished rats was lower than for the control while that for PCM-chow rats was higher than for PCM or control rats. For PCM-TPA rats the biliary clearance was lower than that for the control or PCM-chow rats. When expressed as the clearance per gram of liver, however, the values for PCM and control rats were similar. The biliary clearance per gram of liver for PCM-chow rats was higher than that for PCM rats, while that for PCM-TPA rats was lower.

DISCUSSION

As has previously been reported (14), restricting the amount of chow administered per day to Sprague-Dawley rats results in severe malnutrition. These results illustrate that nutritional repletion with chow or TPA will result in recovery of body and liver weight. The degree of body weight gain depends on the regimen administered while the degree of liver weight (as a ratio to the respective body weight) recovery is equivalent for the different repletion regimens. The differences in dietary composition, however, do not allow comparison of parenteral and enteral routes of repletion.

TABLE 6.—Effect of nutritional status of rats on biliary clearance of MTX

Nutritional status*	Clearance†	Clearance/g of liver
Control	196.2 ± 27.5	20.0 ± 3.0
PCM	96.1 ± 7.3‡	19.2 ± 1.5
PCM-chow	285.9 ± 30.6§	28.8 ± 3.1§
PCM-TPA	109.9 ± 16.3‡,¶	14.2 ± 1.9§,¶

*Control rats were fed ad libitum; final body weight = 282.1 ± 3.4 g.
†Total MTX (μg) excreted divided by AUC.
‡Significantly different ($P < 0.05$) as compared with control rats.
§Significantly different ($P < 0.05$) as compared with PCM rats.
¶Significantly different ($P < 0.05$) as compared with PCM-chow rats.

The increase in the plasma MTX concentration when rats were malnourished would be in part accounted for by the decreased excretion of the drug into the bile. This high plasma concentration might explain the higher toxicity of MTX to similarly malnourished rats (14). These data agree with those recently reported by Mihranian et al (20). These authors reported an increased plasma MTX concentration when rats were fed a protein-free diet which was decreased when similarly malnourished rats were refed chow. Similar results in malnourished cancer patients who were given either oral or parenteral hyperalimentation might have significant effects on toxicity and antitumor activity of MTX and other drugs.

These findings confirm previous reports (21,22) that MTX is transported from plasma to bile by a carrier-mediated process in the rat liver, the biliary concentration of MTX in these studies being much greater than that in the plasma at a given time. The biliary clearance suggests that nutritional repletion with chow and TPA has significant but opposite effects on the hepatobiliary excretion of MTX. The biliary clearance of MTX by the control and PCM rats was similarly related to the liver size while that for PCM-chow and PCM-TPA rats was not. This would suggest that some change in transport of MTX occurs during repletion and alters the clearance of MTX by the rat liver. The excretion of MTX via other routes would have to be examined before the overall effect of malnutrition and nutritional repletion can be determined.

REFERENCES

1. Donaldson SS, and Lenon RA. Alterations of nutritional status—impact of chemotherapy and radiation therapy. Cancer 43:2036–2052, 1979.
2. Warren S. The immediate causes of death in cancer. Am J Med Sci 184:610–615, 1932.
3. Lanzotti VJ, Thomas DR, Bogle LE, et al. Survival with inoperable lung cancer: an integration of prognostic variables based on simple clinical criteria. Cancer 39:303–313, 1977.
4. Issel BF, Valdivieso M, Zaren HA, et al. Protection against chemotherapy toxicity by iv hyperalimentation. Cancer Treat Rep 62:1139–1143, 1978.
5. Souchon EA, Copeland EM, Watson P, et al. Intravenous hyperalimentation as an adjunct to cancer chemotherapy with 5-fluorouracil. J Surg Res 18:451–454, 1975.
6. Steiger E, Oram-Smith J, Miller E, et al. Effects of nutrition on tumor growth and tolerance to chemotherapy. J Surg Res 18:455–461, 1975.
7. Gram TE, Guarino AM, Schroeder DH, et al. The effect of starvation on the kinetics of drug oxidation by hepatic microsomal enzymes from male and female rat. J Pharmacol Exp Ther 175:12–21, 1970.
8. Hartshorn RD, Demers LM, Sultatos LS, et al. Effects of chronic parenteral carbohydrate administration on hepatic drug metabolism in the rat. Pharmacology 18:103–111, 1979.
9. Peters MA, and Strother A. A study of some possible mechanisms by which glucose inhibits drug metabolism in vivo and in vitro. J Pharmacol Exp Ther 180:151–157, 1972.
10. Alvares AP, Anderson KE, Conney AH, et al. Interactions between nutritional factors and drug biotransformations in man. Proc Natl Acad Sci USA 73:2501–2504, 1976.
11. Hopkins GJ, and West CE. Effect of dietary fats on pentabarbitone-induced sleeping times and hepatic microsomal cytochrome P-450 in rats. Lipids 11:736–740, 1976.
12. Norred WP, and Wade E. Dietary fatty acid-induced alterations of hepatic microsomal drug metabolism. Biochem Pharmacol 21:2887–2897, 1972.
13. Krishraswamy K. Drug metabolism and pharmacokinetics in malnutrition. Clin Pharmacol 3:216–240, 1978.
14. Grossie VB, Jr, Ho DHW, and Loo TL. Effect of malnutrition on methotrexate toxicity and tissue levels of dihydrofolate reductase in the rat. Cancer Treat Rep 66:85–89, 1982.
15. Henderson ES, Adamson RH, Denham C, et al. The fate of tritiated methotrexate I. Absorption, excretion, and distribution in mice, rats, dogs, and monkeys. Cancer Res 24:1008–1017, 1965.
16. Calvert AH, Bondy PK, and Harrap KR. Some observations on the human pharmacology of methotrexate. Cancer Treat Rep 61:1647–1656, 1977.
17. Creaven PJ, Hansen HH, Alford DA, et al. Methotrexate in liver and bile after intravenous dosage in man. Br J Cancer 28:589–591, 1973.
18. Steiger E, Vars HM, and Dudrick SJ. A technique for long term intravenous feeding in unrestrained rats. Ann Surg 104:330–335, 1972.
19. Oliverio VT. Chromatographic separation and purification of folic acid analogs. Anal Chem 33:263–265, 1961.
20. Mihranian MH, Daly MD, Wang YM, et al. Methotrexate (MTX) pharmacokinetics: effect of protein depletion and repletion. *In* Proceedings of the Association of Academic Surgeons 16th Annual Meeting, Nov 7–10, 1982, p 84.
21. Strum WB, and Liem HH. Hepatic uptake, intracellular protein binding and biliary excretion of amethopterin. Biochem Pharmacol 26:1236–1240, 1977.
22. Kates RE, and Tozar TN. Biliary secretion of methotrexate in rats and its inhibition by probenacid. J Pharm Sci 65:1345–1352 1976.

Effect of Pretreatment With Phenobarbital or SKF 525A on the Toxicity and Antitumor Activity of Lomustine[1,2,3]

Dietmar W. Siemann[4,5,*]

Previous investigations have shown that the combination of the conventional chemotherapeutic agent lomustine (CCNU) and the nitroimidazole radiation sensitizer misonidazole can lead to an improved therapeutic result. To study whether altered CCNU metabolism plays a role in this chemopotentiation, mice were treated with known modifiers of hepatic microsomal enzymes prior to CCNU exposure, and tumor response and systemic toxicity were assessed. KHT sarcoma-bearing C3H/HeJ mice were pretreated with either (*a*) five daily doses of phenobarbital (80 mg/kg) to induce the microsomal enzymes and then CCNU 48 hours later or (*b*) a 50-mg/kg dose of the microsomal enzyme inhibitor SKF 525A 1 hour before CCNU. Tumor response and normal tissue toxicity following treatment were measured using a regrowth delay and 30-day lethality assay, respectively. Phenobarbital pretreatment reduced both the antitumor efficacy and toxicity of CCNU (factor of ~ 0.8), while SKF 525A increased the effect of CCNU in both cases (factor of ~ 1.6). Thus, unlike pretreatments with the sensitizer misonidazole, pretreatments with phenobarbital or SKF 525A did not result in a therapeutic advantage for CCNU. [Cancer Treat Rep 67:259–265, 1983]

Recent investigations have demonstrated that the in vivo efficacy of certain alkylating antitumor agents can be enhanced effectively by hypoxic cell radiation sensitizers such as the 2-nitroimidazole misonidazole (MISO) (1,2). The nitrosourea lomustine (CCNU) is one chemotherapeutic agent which has shown increased antitumor activity when combined with MISO (1,3–6). More importantly, however, some studies utilizing the CCNU plus MISO combination have demonstrated less enhancement of toxicity in normal tissues such as bone marrow and gut (usually factors of ~ 1.0–1.4) than of the antitumor effects (usually factors of ~ 1.7–2.4), thereby indicating that an improved therapeutic result may be achieved by combining these two agents (1–4,6).

One possible mechanism for the enhancement of the tumor response to alkylating agents by nitroimidazole sensitizers is that the sensitizer interferes with the metabolism and bioavailability of the chemotherapeutic agent (3–5,7,8). When CCNU and MISO are combined, the possibility that this can occur clearly exists. CCNU not only decays spontaneously but is rapidly hydroxylated by the liver microsomal mixed-function oxidase system. This rate of hydroxylation exceeds the rate of chemical dissociation (9–11). Metabolism of the 2-nitroimidazole radiation sensitizer MISO to its *O*-demethylated metabolite Ro-05-9963 is also believed to occur via hepatic microsomal mixed-function oxidases (12–15). Consequently, it is conceivable that MISO may lead to chemopotentiation by competing with CCNU for microsomal enzymes and decreasing CCNU metabolism. Recent findings, which indicated that pretreating animals with the mixed-function oxidase inhibitor SKF 525A could increase the antitumor activity of CCNU to as large a degree as MISO (5), appear to support this point of view. However, as discussed earlier, CCNU-MISO combinations not only increase the antitumor response but also may lead to a therapeutic advantage over the single agent. The effect of pretreating mice with the

[1]Received July 8, 1982; revised Dec 3, 1982; accepted Dec 28, 1982.

[2]Supported by Public Health Service grants CA-11051, CA-20329, and CA-11198 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Presented in part at the 30th Annual Meeting of the Radiation Research Society, Salt Lake City, UT, Apr 1982.

[4]Experimental Therapeutics Division and Department of Radiation Biology and Biophysics, University of Rochester Cancer Center, NY.

[5]I thank Sharon Morrissey and Karen Wolf for their excellent technical assistance and Dr. Paul Workman for excellent discussions.

Reprint requests to: Dietmar W. Siemann, PhD, Experimental Therapeutics Division, University of Rochester Cancer Center, 601 Elmwood Ave, Box 704, Rochester, NY 14642.

known mixed-function oxidase modifiers phenobarbital and SKF 525A (16–18) on both the resultant antitumor efficacy and the degree of normal tissue toxicity of CCNU therefore was evaluated in the present series of experiments. The aim of these investigations was to assess whether non-nitroimidazole agents could mimic the therapeutic gain achieved with MISO.

MATERIALS AND METHODS

Animals and Tumors

Female C3H/HeJ mice (8–14 weeks old), obtained from The Jackson Laboratories, Bar Harbor, ME, were used in all experiments. KHT sarcoma cells (19) were prepared using a dissociation procedure previously described (20) and passaged in vivo every 2 weeks. Tumor cells were transplanted into the left hind legs of recipient animals by injecting 2×10^5 cells im. By 7 days the tumors had grown to a size equivalent to a weight of 0.2–0.3 g (*see* Tumor Response section). The mice were then randomly allocated to a number of groups and treated or kept as controls.

Drug Treatments

CCNU was obtained from Dr. Robert Engle of the Pharmaceutical Resources Branch, National Cancer Institute (Bethesda, MD). A 10-mg sample of CCNU was first dissolved in 1 ml of absolute ethanol and then, immediately prior to injecting the mice, 9 ml of a 0.3% solution of hydroxypropyl cellulose in sterile water was added to this CCNU stock solution. All injections were given ip and on an animal weight basis.

SKF 525A was obtained as a gift from Smith, Kline and French Laboratories (Philadelphia, PA). SKF 525A and sodium phenobarbital were freshly prepared prior to use by dissolving them in saline. These agents were injected ip at 0.01 ml/g of body weight.

Sodium pentobarbital was obtained as a solution and prior to injection it was diluted with saline and administered at a dose of 60 mg/kg at 0.01 ml/g of body weight.

Treatment Schedules

The same treatment schedules were employed in both the tumor response and normal tissue toxicity studies. To inhibit liver microsomal enzymes, a single dose of 50 mg/kg of SKF 525A was administered 60 minutes prior to the chemotherapeutic agent in a manner similar to that described by others (14,21). To induce the microsomal drug-metabolizing enzymes, mice received a dose of 80 mg/kg of phenobarbital once daily on Days 1–5 (14); CCNU subsequently was administered on Day 7.

Pentobarbital Sleep Time

Mice were injected with SKF 525A and phenobarbital as described above. The effects of SKF 525A and phenobarbital pretreatments on the pentobarbital sleep times then were determined on Day 7. Control or pretreated mice were injected with a single dose (60 mg/kg) of pentobarbital and the time for the mice to regain the righting reflex was recorded (12,13,22).

Tumor Response

Tumor response to CCNU in the presence or absence of pretreatments with SKF 525A or phenobarbital was determined using a tumor growth delay assay. One week after tumor cell inoculation mice with equal-sized tumors were selected and randomly allocated to the treatment or control groups. Tumor sizes were determined by passing the tumor-bearing legs through a plastic plate with holes of increasing diameter (23). The smallest hole the tumor-bearing leg would pass through was recorded and converted to a tumor weight using a calibration curve obtained by excising and weighing the tumors of tumor-bearing legs of various sizes (23,24). Following treatment, the tumors were measured daily and the time for each tumor in each group to grow to five times the starting size was recorded. Tumor growth delay was then determined by measuring the differences between the times required by treated and control tumors to grow to a weight equal to five times the weight at Day 0.

Normal Tissue Toxicity

In the normal tissue toxicity studies, the drugs were prepared and the animals were treated as in the tumor response evaluations. Toxicity for CCNU alone and CCNU plus SKF 525A or phenobarbital was determined by recording the number of mice that died during a 30-day posttreatment period. From these data, the LD50/30 values (doses lethal to 50% of the mice in 30 days) and 95% confidence intervals for the various treatments were calculated using a logit bioassay (25) and Scheffe's discrimination interval (26). Non-tumor-bearing C3H/HeJ mice were used because tumor regrowth made it impossible to perform these studies on tumor-bearing mice.

RESULTS

Effect of Pretreatment With Phenobarbital or SKF 525A on Pentobarbital Sleep Time

To determine the extent of induction or inhibition of the liver microsomal mixed-function oxidases by phenobarbital and SKF 525A, respectively, the pentobar-

TABLE 1.—Effect of pretreatment with phenobarbital or SKF 525A on the pentobarbital sleep time in KHT sarcoma-bearing female C3H/HeJ mice*

Treatment	Sleep time in mins (mean ± SD)	% of untreated control
Experiment 1		
Pentobarbital	57.3 ± 5.2	100
Phenobarbital-pentobarbital	20.9 ± 1.0	36.5
Experiment 2		
Pentobarbital	82.4 ± 5.4	100
Phenobarbital-pentobarbital	29.4 ± 2.2	35.7
SKF 525A-pentobarbital	1235.1 ± 29.7	1498.9

*Groups of 6–10 mice were treated with (*a*) a single dose of phenobarbital (80 mg/kg) once daily on Days 1–5 followed 48 hrs later by a 60-mg/kg dose of pentobarbital, (*b*) a single dose of SKF 525A (50 mg/kg) 1 hr before a 60-mg/kg dose of pentobarbital, or (*c*) a 60-mg/kg dose of pentobarbital without pretreatment.

bital sleep times were measured (12,13,18,22). Table 1 shows some representative data. The results indicate that phenobarbital pretreatment significantly reduced the pentobarbital sleep time while SKF 525A had the reverse effect.

Effect of Pretreatment With Phenobarbital or SKF 525A on the Tumor Response to CCNU

The antitumor efficacy of CCNU in the presence or absence of pretreatments with phenobarbital or SKF 525A was assessed by a growth delay assay. Groups of seven to nine mice were treated with each regimen and tumor weight was calculated for each mouse as a function of time after treatment. The doses of SKF 525A and phenobarbital employed, when administered alone, had no effect on tumor growth, but they did alter the antitumor activity of CCNU. This is illustrated in figure 1, which shows that when SKF 525A preceded CCNU treatment, the resultant tumor growth delay (using the time to grow to 1.0 g as the endpoint) increased from ~ 5 to ~ 11.5 days. Phenobarbital pretreatment, on the other hand, reduced the tumor growth delay to ~ 2.5 days.

Similar studies were performed using a range of CCNU doses; figures 2 and 3 show the pooled results of the effects of pretreatments with SKF 525A or phenobarbital, respectively, on CCNU efficacy. In these two

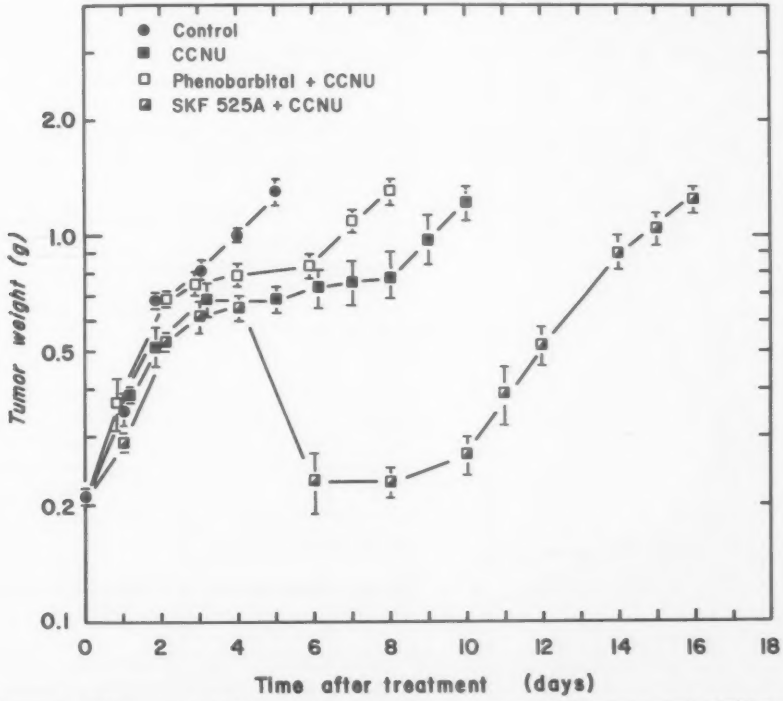


FIGURE 1.—KHT sarcoma weight as a function of time after no treatment (solid circles), a single CCNU dose of 15 mg/kg (solid squares), or pretreatments of phenobarbital (open squares) or SKF 525A (harlequin squares) administered prior to 15 mg/kg of CCNU. Phenobarbital (80 mg/kg) was given daily on Days 1–5 and CCNU was given 48 hrs later. SKF 525A (50 mg/kg) preceded CCNU by 1 hr. Tumor response to a given treatment was obtained by converting from a diameter measurement to a tumor weight using a calibration curve (*see* Methods section). The data shown are the mean ± SE of groups of 7–9 mice.

figures the median rather than the mean times to grow to five times the starting weight are illustrated. This is because with the more effective treatments employed, a considerable range of responses was observed and some mice had to be killed for humane reasons before the tumors of others in the same group had reached the desired endpoint. The median tumor response therefore was believed to be more representative. The results in figure 2 show that pretreatment with SKF 525A significantly enhanced the tumor growth delay at every dose of CCNU. Conversely, the phenobarbital pretreatment regimen reduced the tumor response to CCNU (fig 3).

From the data illustrated in figures 2 and 3 the enhancement ratios (ERs), defined as the dose of CCNU alone divided by the dose of CCNU plus pretreatment required for a given tumor effect, were calculated for various doses of CCNU alone (table 2). For SKF 525A pretreatment, a constant enhancement in the tumor response (ER ~ 1.6) was observed. Pretreatment with phenobarbital reduced the efficacy of CCNU and consequently led to ERs < 1.0.

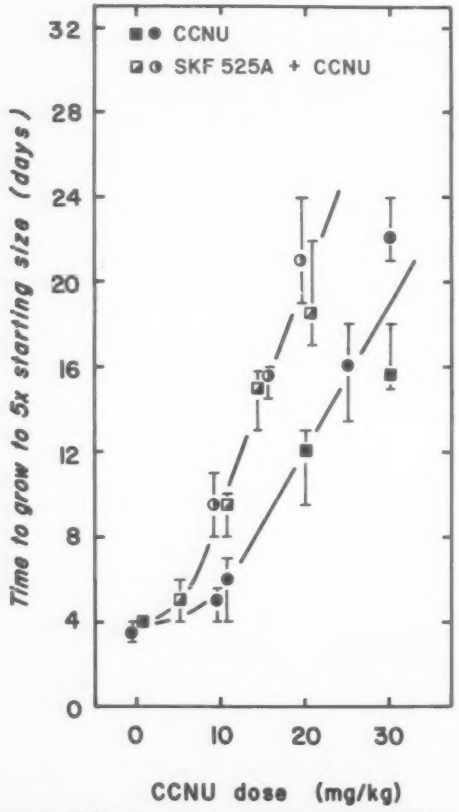


FIGURE 2.—Median time to grow to 5 times the starting weight as a function of the CCNU dose for KHT sarcoma-bearing mice treated with CCNU alone (solid symbols) or SKF 525A + CCNU (harlequin symbols). SKF 525A (50 mg/kg) was administered 1 hr prior to CCNU. The results shown are the median tumor responses (± 98% confidence limits) of 7–10 mice. Confidence intervals about the median were calculated using nonparametric statistics (27). Differences in the shapes of symbols represent different experiments.

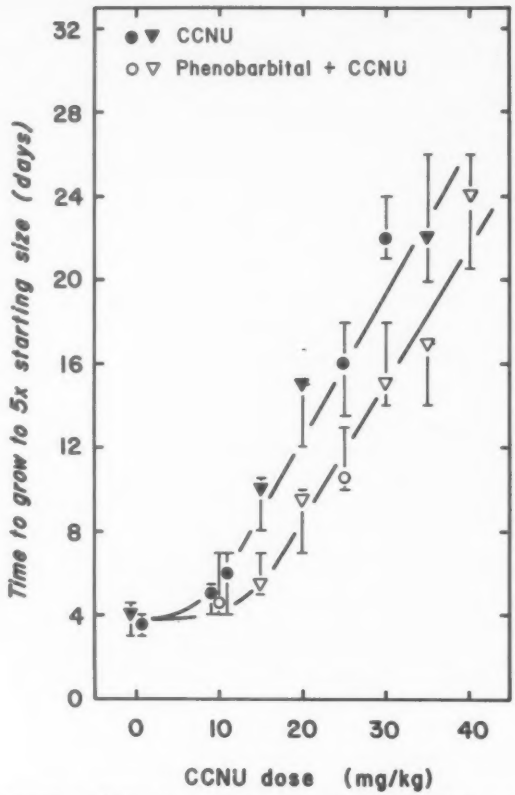


FIGURE 3.—Median time to reach 5 times the starting weight as a function of the CCNU dose for KHT sarcoma-bearing mice treated with CCNU alone (solid symbols) or phenobarbital + CCNU (open symbols). Phenobarbital was administered as described in fig 1. Data shown are as described in fig 2.

Effect of Pretreatment With Phenobarbital or SKF 525A on the Toxicity of CCNU

For comparison with the antitumor activity studies described above, experiments were performed to evaluate to what degree the phenobarbital and SKF 525A pretreatment regimens would modify the lethal toxicity in female C3H/HeJ mice treated with single doses of CCNU. Previous investigations (3,21,28) have shown that most animals die from gut toxicity within 5–9 days

TABLE 2.—ERs* for KHT sarcoma-bearing mice pretreated with phenobarbital† or SKF 525A‡

Dose of CCNU alone (mg/kg)	Dose of CCNU (mg/kg) after pretreatment		ER	
	Phenobarbital pretreatment	SKF 525A pretreatment	Phenobarbital pretreatment	SKF 525A pretreatment
10	15	6.5	0.7	1.5
15	20.5	9.5	0.7	1.6
20	26	12.5	0.8	1.6
25	31.5	15.5	0.8	1.6
30	36.5	19	0.8	1.6
35	42	—	0.8	—

*Dose of CCNU alone divided by the dose of CCNU + pretreatment required for a given tumor effect.
†Phenobarbital was given as daily 80-mg/kg doses on Days 1–5 followed 48 hrs later by CCNU.
‡SKF 525A was administered as a single dose of 50 mg/kg 1 hr before CCNU.

following single-dose nitrosourea administration. However, since CCNU also is toxic to the bone marrow (4,6,28) and since a few animal deaths occurred at times usually associated with bone marrow toxicity, the mice were observed for 30 days after treatment to include both the gut and bone marrow toxicity syndromes.

The results (fig 4) show that pretreating mice with SKF 525A before CCNU increased the lethal toxicity of CCNU and consequently reduced the LD50/30 from 52 (50–54) (95% confidence interval) to 33 (30–36) mg/kg. Administering phenobarbital prior to CCNU protected the mice from the toxic effects of CCNU and increased the LD50/30 value to 64 (62–66) mg/kg. The combination of SKF 525A or phenobarbital with a range of single doses of CCNU therefore resulted in systemic toxicity ERs of ~ 1.6 and ~ 0.8, respectively.

DISCUSSION

Previous investigations have shown that when the nitroimidazole hypoxic cell sensitizer MISO is added to CCNU treatments an improved therapeutic result may be achieved (1–3,6). Since both agents are metabolized by liver microsomes, the present studies were aimed at evaluating whether a possible mechanism for this therapeutic gain might be the interference by MISO with the metabolism of CCNU. To test this hypothesis known non-nitroimidazole modifiers of the hepatic mixed-

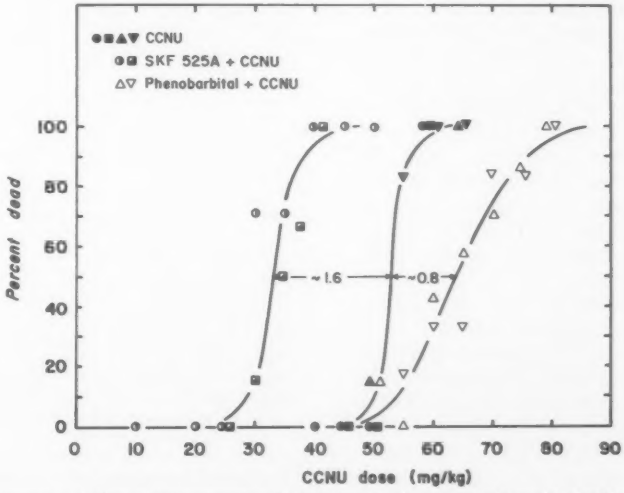


FIGURE 4.—Effect of pretreatments with SKF 525A (harlequin symbols) or phenobarbital (open symbols) on the 30-day survival of non-tumor-bearing C3H/HeJ mice treated with CCNU. SKF 525A and phenobarbital were administered as described in fig 1. Solid symbols are the results for mice receiving CCNU without pretreatment. Differences in the shapes of symbols represent different experiments. Each data point represents 6–12 mice.

function oxidase system were combined with CCNU and the effect of such pretreatments on both the antitumor activity and toxicity of CCNU was examined.

The present data (table 1) indicate that pretreating mice with phenobarbital or SKF 525A altered the rates of liver metabolism, as assessed by barbiturate sleep times, to an extent similar to that previously described by others (12,13,17,18). Such pretreatments also increased or decreased both the antitumor efficacy and the toxicity of CCNU. A single dose of SKF 525A administered prior to single-dose CCNU exposures significantly enhanced the resultant tumor response (fig 2) and systemic toxicity (fig 4) of this agent. Repeated phenobarbital administrations had the opposite effect, making the drug both less effective (fig 3) and less toxic (fig 4). These results likely were a consequence of a decrease or increase in the rate of mixed-function oxidase metabolism of CCNU in the presence of SKF 525A and phenobarbital, respectively.

Previously, Levin et al (29) found similar results in 9L brain tumor-bearing rats given carmustine (BCNU) following pretreatment with phenobarbital. These authors showed that the phenobarbital pretreatment led to decreases in the plasma concentration, antitumor efficacy, and systemic toxicity of BCNU due to increased liver metabolism of the chemotherapeutic agent. Compared to BCNU, a somewhat smaller effect was seen with CCNU. The present findings also are consistent with those of Klubes et al (21) who reported on the effects of pretreating mice with SKF 525A and phenobarbital prior to semustine (MeCCNU) administration. In their investigations, increases in lifespan but also systemic toxicity were observed in L1210-bearing $B6D2F_1$ mice treated with SKF 525A prior to MeCCNU. Conversely, when liver metabolism was induced by pre-exposing mice to phenobarbital, both the toxicity and antitumor activity of MeCCNU were reduced (21). Those findings are qualitatively similar to the ones shown in figures 2–4. All of these results suggest that in cancer patients receiving agents which may alter liver enzyme metabolism while undergoing nitrosourea therapy, antitumor efficacy or normal tissue complications may be influenced.

CCNU is rapidly metabolized by liver microsomal enzymes. This is achieved primarily by hydroxylation of the cyclohexyl ring which leaves the chloroethyl moiety intact (30–32). Studies evaluating the tumoricidal effects of the hydroxylated products have shown them to have similar efficacies to the parent compound (11,31). The present data indicate that SKF 525A and phenobarbital had opposing effects on the biologic activity of CCNU, probably because the former inhibits and the latter enhances the metabolism of CCNU. The findings therefore imply that the extent of metabolism of the parent compound determines the therapeutic dose of CCNU.

As was observed previously for MeCCNU by Klubes et al (21), modifying hepatic microsomal enzyme activities also did not yield a therapeutic advantage for CCNU in the present study. This was a consequence of the findings that phenobarbital and SKF 525A pretreatments each alter both the antitumor activity and normal tissue toxicity to the same extent. Phenobarbital pretreatment reduced the systemic toxicity of CCNU by a factor of ~ 0.8 but also decreased the agent's effectiveness to the same degree (fig 3 vs fig 4). Enhanced tumor responses (ER ~ 1.6) were observed when SKF 525A preceded CCNU exposure but only at the cost of a similar increase in host toxicity (fig 2 vs fig 4).

Despite this failure to obtain a therapeutic advantage when CCNU and SKF 525A or phenobarbital were combined, the present investigations do not rule out the possibility that pharmacokinetic modification contributes to the potentiation of the activity of CCNU by MISO. Since nitroimidazoles such as MISO are potent inhibitors of microsomal cytochrome P-450 activity (33), further investigations into the pharmacokinetic mechanism for CCNU potentiation by MISO are required. To evaluate this more directly, studies are currently underway to measure, using high-performance liquid chromatographic techniques, the effects of phenobarbital, SKF 525A, or MISO pretreatments on CCNU plasma pharmacokinetics.

REFERENCES

1. McNally N. Enhancement of chemotherapy. Int J Radiat Oncol Biol Phys 8:593–598, 1982.
2. Siemann DW. Potentiation of chemotherapy by hypoxic cell radiation sensitizers—a review. Int J Radiat Oncol Biol Phys 8:1029–1034, 1982.
3. ———. In vivo combination of misonidazole and the chemotherapeutic agent CCNU. Br J Cancer 43:367–377, 1981.
4. ———. Response of murine tumors to combinations of CCNU with misonidazole and other radiation sensitizers. Br J Cancer 45:272–281, 1982.
5. Workman P, and Twentyman PR. Enhancement by electron-affinic agents of the therapeutic effects of cytotoxic agents against the KHT tumour: structure-activity relationships. Int J Radiat Oncol Biol Phys 8:623–626, 1982.
6. Hirst DG, and Brown JM. The therapeutic potential of misonidazole enhancement of alkylating agent cytotoxicity. Int J Radiat Oncol Biol Phys 8:639–642, 1982.
7. Mulcahy RT, Siemann DW, and Sutherland RM. In vivo response of KHT sarcomas to combination chemotherapy with radiosensitizers and BCNU. Br J Cancer 43:93–99, 1981.
8. Brown JM. On the mechanisms of cytotoxicity and chemosensitization by misonidazole and other nitroimidazoles. Int J Radiat Oncol Biol Phys 8:675–682, 1982.
9. Hilton J, and Walker MD. Hydroxylation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Biochem Pharmacol 24:2153–2158, 1975.
10. Reed RD, and May HE. Alkylation and carbomoylation intermediates from the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). Life Sci 16:1263–1270, 1975.
11. Wheeler GP, Johnston TP, Bowdon BJ, et al. Comparison of the properties of metabolites of CCNU. Biochem Pharmacol 26:2331–2336, 1977.

12. Workman P. Effects of pretreatment with phenobarbitone and phenytoin on the pharmacokinetics and toxicity of misonidazole in mice. Br J Cancer 40:335–353, 1979.
13. ———. Drug interactions with misonidazole: effects of dexamethasone and its derivatives on the pharmacokinetics and toxicity of misonidazole in mice. Biochem Pharmacol 29:2769–2776, 1980.
14. ———. Dose-dependence and related studies on the pharmacokinetics of misonidazole and desmethylmisonidazole in mice. Cancer Chemother Pharmacol 5:27–37, 1980.
15. Shoemaker DD, McManus ME, Hoerauf R, et al. Studies on the *O*-demethylation of misonidazole by rat liver microsomes. Cancer Treat Rep 66:1343–1347, 1982.
16. Anders MW, and Mannering GJ. Kinetics of the inhibition of the *N*-demethylation of ethylmorphine by 2-diethylaminoethyl 2, 2-diphenylvalerate HCl (SKF 525A) and related compounds. Mol Pharmacol 2:319–327, 1966.
17. Marshall WJ, and McLean AEM. The effect of oral phenobarbitone on hepatic microsomal cytochrome P-450 and demethylation activity in rats fed normal and low protein diets. Biochem Pharmacol 18:153–157, 1979.
18. Gerber N, and Arnold K. Studies on the metabolism of diphenylhydantoin in mice. J Pharmacol Exp Ther 167:77–90, 1969.
19. Kallman RF, Silini J, and Van Putten LM. Factors influencing the quantitation of the in vivo survival of cells from solid tumors. JNCI 39:539–549, 1967.
20. Thomson JE, and Rauth AM. An in vitro assay to measure the viability of KHT tumor cells not previously exposed to culture conditions. Radiat Res 58:262–276, 1974.
21. Klubes P, Miller HG, Cerna I, et al. Alterations in the toxicity and antitumor activity of methyl-CCNU in mice following pretreatment with either phenobarbital or SKF 525A. Cancer Treat Rep 63:1901–1907, 1979.
22. Stevenson IH, and Turnbull MR. Hepatic drug-metabolising enzyme activity and duration of hexobarbitone anaesthesia in barbitone-dependent and withdrawn rats. Biochem Pharmacol 17:2297–2306, 1968.
23. Siemann DW, Hill RP, and Bush RS. The importance of pre-irradiation breathing times of oxygen and carbogen (5% CO_2:35% O_2) on the in vivo radiation response of a murine sarcoma. Int J Radiat Oncol Biol Phys 2:903–911, 1977.
24. Siemann DW, and Sutherland RM. In vivo tumor response to single and multiple exposures of adriamycin. Eur J Cancer 16:1433–1440, 1980.
25. Berkson J. Maximum likelihood and minimum χ^2 estimates of the logistic function. J Am Stat Assoc 50:130–162, 1955.
26. Finney DJ. Statistical Methods in Biological Assays. London, Griffin, 1971.
27. Noether J. Introduction to Statistics—A Fresh Approach. Boston, Houghton Mifflin, 1971.
28. Blackett NM, Courtenay VD, and Mayer SM. Differential sensitivity of colony-forming cells of hemopoietic tissue, Lewis lung carcinoma, and B16 melanoma to three nitrosoureas. Cancer Chemother Rep 59:929–933, 1975.
29. Levin VA, Stearns J, Byrd A, et al. The effect of phenobarbital pretreatment on the antitumor activity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl-1-nitrosourea (PCNU), and on the plasma pharmacokinetics and biotransformation of BCNU. J Pharmacol Exp Ther 208:1–6, 1979.
30. May HE, Boose R, and Reed DJ. The hydroxylation of the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) by rat liver microsomes. Biochem Biophys Res Comm 57:426–433, 1974.
31. Johnston TP, McCaleb S, and Montgomery JA. Synthesis and biological evaluations of major metabolites of *N*-(2-chloroethyl)-*N*-cyclohexyl-*N*-nitrosourea. J Med Chem 18:634–637, 1975.
32. Kohlhepp SJ, May HE, and Reed DJ. Urinary metabolites of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea. Drug Metab Dispos 9:135–141, 1981.
33. Workman P, Twentyman PR, Lee FYF, et al. Drug metabolism and chemosensitization: nitroimidazoles as inhibitors of drug metabolism. Biochem Pharmacol, 1982. In press.

Biochemical Pharmacology and Toxicology of Formycin Alone and in Combination With 2′-Deoxycoformycin (Pentostatin)[1,2]

Ellen N. Spremulli,[3,4,*] Gerald W. Crabtree,[4] Daniel L. Dexter,[3,4] Shih Hsi Chu,[4] Diane M. Farineau,[3] Lucy Y. Ghoda,[4] D. Lynn McGowan,[4] Israel Diamond,[3,4] Robert E. Parks, Jr,[4] and Paul Calabresi[3,4]

The toxicology and pharmacology of formycin both as a single agent and combined with the adenosine deaminase inhibitor 2′-deoxycoformycin (dCF) were examined in outbred Swiss mice heterozygous for the nude gene (nu/+). The LD10 for formycin alone given on a daily × 5 schedule was 21 mg/kg. When the animals were pretreated with 1 mg/kg of dCF 1 hour prior to each dose of formycin, toxicity was approximately doubled, ie, LD10 was reduced to 10 mg/kg. Death was associated with hepatic toxicity in both treatment regimens; suppression of leukocyte counts was mild except at doses greater than the LD10. Formycin nucleotides were detected by high-performance liquid chromatography in the livers of mice treated with formycin either alone or combined with dCF. When isolated rat hepatocytes were incubated for 2 hours with either formycin or dCF plus formycin, analog nucleotides accumulated in the cells. Cellular ATP decreased to below the limits of detection, whereas a large peak corresponding to formycin-5′-triphosphate was present. This replacement of cellular ATP by formycin-5′-triphosphate may help explain the hepatic toxicity observed. [Cancer Treat Rep 67:267–274, 1983]

Formycin and its deamination product, the inosine analog formycin B, were isolated by Hori et al (1) from a culture of *Nocardia interforma*. Current evidence indicates that formycin, an adenosine analog which possesses an unusual C-C glycosidic bond, must be phosphorylated to the 5′-triphosphate nucleotide to show antineoplastic activity (2–5). The synthesis of formycin-5′-triphosphate from formycin requires the sequential actions of the enzymes adenosine kinase, adenylate kinase, and nucleoside diphosphokinase. Formycin is readily deaminated by adenosine deaminase (ADA) to yield formycin B (4,6), which is further metabolized by liver aldehyde oxidase to produce oxoformycin (7–9). In studies on the biochemical effects of formycin in Ehrlich ascites tumor cells, Henderson et al (3) determined that de novo purine synthesis was blocked by formycin as a consequence of inhibition of the synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP). PRPP synthesis was presumably inhibited by high levels of formycin polyphosphates at the level of the phosphoribose pyrophosphokinase (PRPP synthetase) reaction, since this enzyme is under strong negative feedback control by ADP and ATP (10,11).

Ikehara et al (12,13) found that formycin-5′-triphosphate could be incorporated into polynucleotides by *Escherichia coli* DNA-dependent RNA polymerase and that the analog nucleotide could replace ATP in such polymers. Furthermore, the coding properties of ribopolynucleotides that contained formycin were the same as those of ribopolynucleotides that contained adenosine (12). Ward et al (14–17) performed a series of studies showing that formycin-containing polymers had a number of unusual properties when compared with polymers that contain adenosine. Polyformycin resem-

[1]Received July 19, 1982; revised Dec 3, 1982; accepted Dec 28, 1982.

[2]Supported by Public Health Service grants CA-07340 and CA-26218, and CA-13943 and CA-20892 (a collaborative study of the Roger Williams Cancer Center) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine (*E. N. Spremulli, D. L. Dexter, D. M. Farineau*, and *P. Calabresi*) and Pathology (*I. Diamond*), Roger Williams General Hospital, Providence, RI.

[4]Sections of Biochemical Pharmacology (*G. W. Crabtree, S. H. Chu, L. Y. Ghoda, D. L. McGowan*, and *R. E. Parks, Jr*), Medicine (*E. N. Spremulli, D. L. Dexter*, and *P. Calabresi*), and Pathology (*I. Diamond*), Division of Biology and Medicine, Brown University, Providence, RI.

*Reprint requests to: Ellen N. Spremulli, MD, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Ave, Providence, RI 02908.

bled polycytosine rather than polyadenosine in its reaction with bovine pancreatic ribonuclease A (14). The coding properties of a copolymer of formycin monophosphate and uridine monophosphate were analogous to those for the corresponding adenosine-uridine polymers, except that the formycin-containing copolymer did not code for polylysine synthesis (15). In addition, the homopolymer polyformycin, unlike polyadenosine, was unable to act as a template for polylysine synthesis (15). Other properties of formycin polymers included ultraviolet hyperchromism, fluorescence, and modified thermostability (16,17). In addition, various steps of DNA transcription such as chain initiation and the release of newly synthesized RNA chains from the ternary transcription complex are inhibited by formycin triphosphate in comparison to the same processes using natural purine nucleotides (18). Many of these properties were thought to result from conformation transitions which were made possible by the greater freedom of rotation of the C-C bond of formycin as compared to the N-C bond of adenosine (16).

Previous work revealed that formycin has activity as both an antiviral (19,20) and an antineoplastic (1,6,21–23) agent. In contrast, formycin B has no appreciable antineoplastic activity (6,22,23), and its antibacterial activity is limited to *Xanthomonos oryzae* (6). Formycin B does, however, have antiviral (19,20) and immunosuppressive activity (24,25) that cannot be explained completely on the basis of its inhibitory activity with purine nucleoside phosphorylase (26). Formycin B has also been proposed to be an inhibitor of the synthesis of polyadenosine diphosphoribose (27). Variations in the content of this polymer may play a role in cell division and/or gene expression (27).

The availability of 2′-deoxycoformycin (dCF, Pentostatin), a tight binding inhibitor of ADA (28,29), which prevents the deamination of formycin, has renewed interest in formycin as a potential antineoplastic agent. Previous work has shown that the related tight binding inhibitor of ADA, coformycin, can potentiate the tumor growth inhibitory properties of formycin (4) and that dCF greatly increases the cytotoxicity of formycin against human colon and pancreas tumor cell lines in vitro (30,31). On the basis of these results we have undertaken a detailed toxicologic evaluation of formycin both as a single agent and in combination with dCF in mice, prior to performing more extensive studies of this combination against human tumors grown as xenografts in athymic mice.

METHODS

Formycin was either purchased from the Sigma Chemical Co (St. Louis, MO) or obtained from the Drug Synthesis Branch, National Cancer Institute (NCI; Bethesda, MD) and dissolved in physiologic saline at a concentration of 1 or 2 mg/ml for use in toxicology studies. dCF was also obtained from NCI and was dissolved in saline at a concentration of 0.1 mg/ml prior to use.

Mice used in this study were male and female outbred Swiss heterozygous for the nude gene (nu/+), bred at the Roger Williams Cancer Center Animal Care Facility. Animals were used when they were between 8 and 20 weeks of age and weighed 20–40 g. Food and water were available ad libitum during the experiments. Males were housed individually and females were housed 13 per cage.

Rats (CD_1 albino; 250–300 g), from which hepatocytes were isolated, were purchased from Charles River Breeding Laboratories (Wilmington, MA). These animals were kept in the Animal Care Facility at Brown University and given food and water ad libitum.

The lethal dose for 10% of the animals (LD10) and the lethal dose for 50% of the animals (LD50) were determined by treating mice on a once daily for 5 days (qd × 5) schedule. When formycin was used in combination with dCF, the dCF was given at a dose of 1 mg/kg each day 1 hour prior to formycin administration. Treatment groups consisted of 13 male and 13 female mice, and all injections were given ip. All animals were weighed on Days 6 and 14; blood cell counts and blood chemistries were obtained on Days 6 and 14. Cages were checked twice daily for deaths. A complete autopsy with histologic examination of the tissues was done on at least one animal per group on Days 6 and 14.

Peripheral leukocyte counts were determined using a Becton Dickinson wbc counting kit on two animals on Day 6 and again on Day 14, and the counts obtained on each day were averaged. Glucose, BUN, creatinine, SGOT, and bilirubin determinations were performed in the clinical chemistry laboratory at Roger Williams General Hospital.

Autopsies were performed after cervical dislocation. All organs including brain were examined both grossly and microscopically. Slides were prepared after fixation in 10% glutaraldehyde and Formalin and were stained with hematoxylin and eosin. Selected livers were quick-frozen and stained with periodic acid-Schiff (PAS) and Sudan III fat stain for glycogen and fat. In addition, mice were treated with formycin (32.5 mg/kg) or saline, deprived of food for 24 hours, and killed, and the livers were examined histologically for glycogen.

In an attempt to explain biochemically the hepatotoxicity observed in animals treated with formycin plus dCF, nucleotide profiles of isolated rat hepatocytes were determined. Hepatocytes were isolated using the two-step collagenase perfusion method of Seglen (32). The perfusion buffer was Krebs-Ringer bicarbonate (pH 7.45) gassed with 95% O_2:5% CO_2. Cells were incubated at 37°C in an atmosphere of 95% O_2:5% CO_2 in medium (pH 7.4) that contained HEPES (50 mM), NaCl (95 mM),

TABLE 1.—Toxicity of formycin alone and in combination with dCF to athymic mice*

Treatment	Toxic dose (mg/kg/day)	
	LD10	LD50†
Formycin alone	21	32 (28.7–35.7)
Formycin + dCF	19	10 (13.1–25.5)

*Mice were treated once per day for 5 successive days by ip injection. When dCF was used in combination with formycin, the dCF, at a dose of 1.0 mg/kg, was administered 1 hr prior to the formycin. Treatment groups consisted of 26 mice (13 male, 13 female). Values listed above were obtained from a log-probit mortality plot.

†95% confidence limits are shown in parentheses.

KCl (45 mM), $CaCl_2 \cdot 2H_2O$ (2.5 mM), KH_2PO_4 (1.2 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM), and glucose (5 mM). When used, dCF was present for 20 minutes before formycin (1 mM final concentration) was added. At appropriate time points (up to 2 hours) after addition of formycin, aliquots of the reaction mixture were removed and cold acid-soluble (perchloric acid, 4% final concentration) extracts were prepared. Following neutralization, the extracts were analyzed by anion-exchange high-performance liquid chromatography (HPLC) as described previously (33). HPLC effluents were monitored at two wavelengths, 254 nm to visualize natural nucleotides and 295 nm for formycin compounds.

The following procedures were carried out to verify the presence of formycin nucleotides in the livers of treated animals. Mice were injected with dCF (1 mg/kg) or saline 1 hour prior to injection of formycin (8.7 or 21.2 mg/kg, respectively). Mice were killed by cervical dislocation at 15, 30, and 45 minutes after injection of formycin, and immediately thereafter laparotomy was performed. Livers were freeze-clamped in situ using stainless-steel clamps which had been previously immersed in a dry ice-ethanol bath. Portions of the frozen livers were placed in chilled (4°C) Dounce homogenizers containing 4% perchloric acid. Following homogenization, the extracts were neutralized and nucleotide profiles determined as described above.

RESULTS

The toxicity of formycin was increased about 1.5- to 2-fold by pretreatment with dCF (table 1). Formycin as a single agent on a qd × 5 schedule had an LD50 of 32 mg/kg/day and an LD10 of 21 mg/kg/day. When dCF was given at a dose of 1 mg/kg/day 1 hour prior to formycin, the LD50 was 19 mg/kg/day and the LD10 was 10 mg/kg/day. No toxicity was observed in animals treated with this dose of dCF alone.

All mice treated with formycin at doses of ≥ 19 mg/kg qd × 5 lost weight by Day 6, and normal weight was regained by Day 14 in all groups except those treated with formycin at 43 mg/kg; the latter group had started to regain weight by Day 14. As would be expected, weight loss was more severe in animals receiving higher doses. Animals treated with 1 mg/kg of dCF prior to injection of formycin lost weight by Day 6 when formycin was given at doses of ≥ 4 mg/kg qd × 5; normal weight was regained by Day 14 in animals receiving < 11 mg/kg qd × 5. Mice receiving dCF (1 mg/kg) alone had no weight loss.

Animals treated with formycin alone did not show leukopenia on Day 6 at doses < 33 mg/kg qd × 5; even at the highest dose used (43 mg/kg) leukopenia was mild. When dCF pretreatment was given, leukocyte

TABLE 2.—Clinical chemistry results from athymic mice treated with formycin alone or in combination with dCF*

Formycin dose (mg/kg/day)	dCF dose (mg/kg/day)	Glucose (mg/dl)	SGOT (mU/ml)	Bilirubin (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
0	0	199	45	0.4	25	0.5
15.0	0	112	90	—	22	0.4
19.5	0	140	98	0.8	32	0.5
24.4	0	103	135	0.5	12	0.3
33.0	0	55	179	1.6	10	0.5
42.9	0	89	324	2.9	24	0.3
0	1	171	54	0.5	29	0.4
3.1	1	159	63	0.5	22	0.4
4.0	1	117	87	0.4	20	0.5
5.2	1	125	77	0.6	39	0.4
6.8	1	132	135	1.5	24	0.4
8.8	1	95	127	2.5	13	0.4
11.5	1	110	129	2.5	35	0.1
15.0	1	104	203	3.8	18	0.4

*Drugs were administered to mice by ip injection once per day for 5 successive days. When dCF was used in combination with formycin, the dCF was given 1 hr prior to the formycin. Treatment groups consisted of 26 mice. Chemistry values were determined on 2 mice/group on Day 6, and values in table are averages of these duplicate determinations. Normal values for the parameters listed above (with ranges) were: glucose, 209 mg/dl (123–280); SGOT, 52 mU/ml (41–61); bilirubin, 0.4 mg/dl (0.1–0.7); BUN, 29 mg/dl (24–32); and creatinine, 0.3 mg/dl (0–0.5).

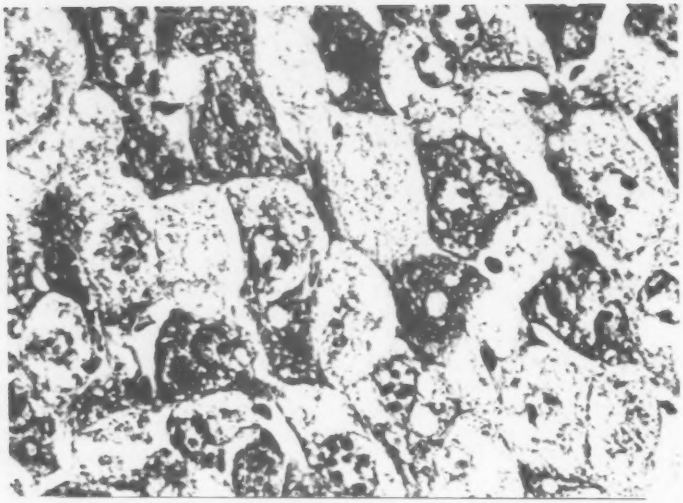


FIGURE 1.—Hematoxylin- and eosin-stained histologic section of liver from a mouse treated ip with formycin on a qd × 5 schedule at a dose of 33 mg/kg/day. Histology was performed on Day 6. Magnification, × 40.

suppression was seen at formycin doses > 4 mg/kg qd × 5; leukocyte counts had recovered to pretreatment values by Day 14 in all groups. At a dose of 1 mg/kg, dCF alone caused mild (< 10%) suppression of total leukocyte counts by Day 6 in 50% of the mice.

Several abnormalities were observed in the blood chemistries determined on Day 6 (table 2). Most notable were elevations in SGOT and bilirubin, which reflect hepatotoxicity. Marked increases in SGOT consistent with acute hepatocellular damage were seen in animals treated with formycin doses > 33 mg/kg qd × 5. Mild elevations of bilirubin were noted in animals treated with 33 and 43 mg/kg of formycin. No alterations in serum creatinine were observed.

Similar alterations were seen in animals treated with the combination of dCF and formycin, with biochemical

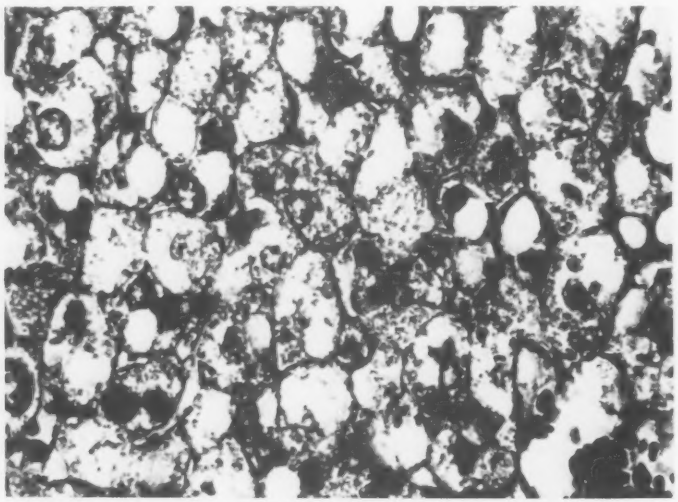


FIGURE 2.—Hematoxylin- and eosin-stained histologic section of liver from a mouse treated with the combination of formycin + dCF. Formycin was administered ip on a qd × 5 schedule at a dose of 21 mg/kg/day 1 hr after ip injection of dCF (1.0 mg/kg/day). Histology was performed on Day 6. Magnification, × 40.

evidence of hepatotoxicity observed at doses of formycin > 8.8 mg/kg qd × 5. As a single agent, dCF at a dose of 1 mg/kg (table 2) did not alter SGOT or bilirubin. On gross postmortem examination, done on Day 6, animals treated with formycin alone at doses of ≥ 15 mg/kg qd × 5 or with > 4 mg/kg qd × 5 of formycin in combination with dCF showed pale, swollen, and smooth livers. Animals killed on Day 14 had grossly normal livers except those treated with formycin at a dose of 25.4 mg/kg qd × 5 if given alone or at doses > 5.2 mg/kg qd × 5 if dCF was also given. In the animals given these latter doses of drugs, abnormal livers were observed. The rest of the gross postmortem examination did not reveal abnormalities.

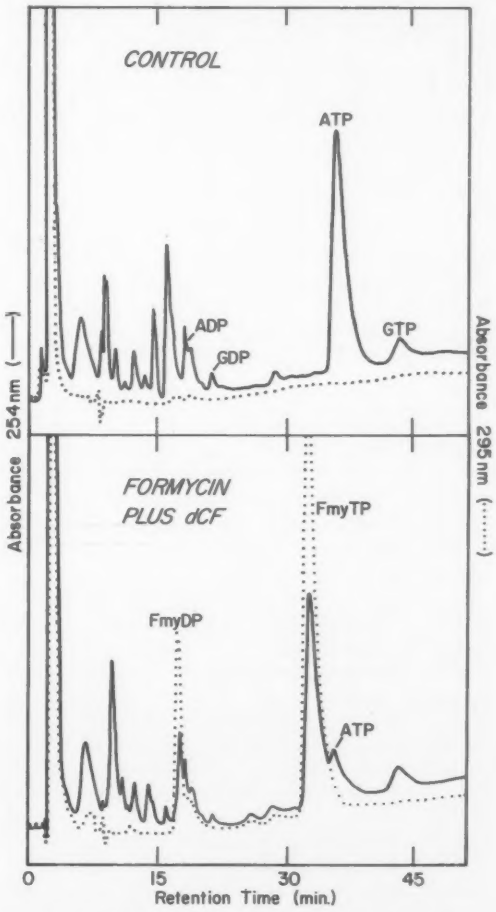


FIGURE 3.—Nucleotide profiles of isolated rat hepatocytes incubated in vitro for 2 hrs in the absence (upper frame) or presence (lower frame) of formycin (1 mM) and dCF (1 μg/ml). The procedures for cell isolation, incubation, extraction, and HPLC are outlined in the Methods section and in ref 33. HPLC column effluents were monitored at 2 wavelengths, 254 nm (solid lines) to detect natural nucleotides and 295 nm (dotted lines) to detect formycin nucleotides. FmyDP and FmyTP denote peaks corresponding to formycin-5′-diphosphate and -triphosphate, respectively.

Histologic examination was performed on hematoxylin- and eosin-stained sections of liver, heart, lung, small and large intestine, esophagus, stomach, pancreas, kidney, bone marrow, spleen, thymus, and brain. The most notable changes were seen in the liver, which contained swollen vacuolated cells, some cytomegaly, and some areas of early regeneration (figs 1 and 2). PAS with and without diastase and Sudan III fat stains indicated the presence of fat in the vacuoles. Histologic examination of the bone marrow correlated well with peripheral leukocyte counts. Marked marrow hypoplasia was seen only in the animals treated with the highest dose of formycin alone, and slight to moderate depression of both peripheral blood granulocytes and erythrocytes was seen in animals treated with formycin doses of 8.8 and 11.5 mg/kg qd × 5 after treatment with dCF. Mild to moderate peritonitis, as evidenced by fibrinous adhesions, occurred in most animals probably secondary to a local irritant effect of formycin.

In an attempt to explain biochemically the hepatotoxicity observed in animals treated with formycin plus dCF, isolated rat hepatocytes were incubated in vitro with formycin alone or in combination with dCF. Figure 3 shows the nucleotide profiles obtained by HPLC of extracts of cells incubated for 2 hours in the absence or presence of the dCF and formycin combination. The levels of formycin-5′-triphosphate that accumulated in these cells in the 2-hour period were 1.5 and 4.0 mM when formycin was used alone and in combination with dCF, respectively. Concurrently, ATP levels fell to about 10% of starting levels (1.5mM) with formycin alone and to undetectable levels when the combination was used. To verify that formycin nucleotides accumulated in livers of treated mice, animals were killed at various times after formycin injection; livers were freeze-clamped, and a portion was excised and extracted with perchloric acid. When nucleotide profiles of the extracts were determined, formycin nucleotides were seen (fig 4). The maximal total concentration of formycin-5′-diphosphate and formycin-5′-triphosphate that accumulated was approximately 0.4 μmol/g of tissue.

DISCUSSION

The LD10 and LD50 values in mice for the adenosine analog formycin, when administered ip once per day for 5 successive days, are 21 and 32 mg/kg/day, respectively. In contrast, the LD50 of formycin when given ip to mice in a single injection is between 250 and 500 mg/kg (22). The discrepancy in the LD50 values is probably explained by the different schedules of drug administration in the two studies.

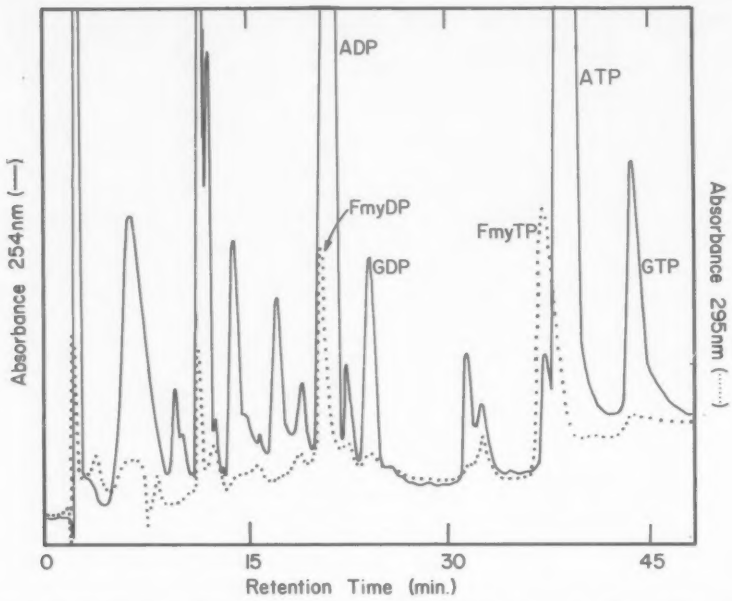


FIGURE 4.—Nucleotide profile of liver removed from a mouse 45 mins after injection of formycin (8.7 mg/kg). The formycin was preceded 1 hr by administration of dCF (1 mg/kg). *See* legend to fig 3 for additional details.

In the present study, the major toxic effects of formycin were confined to the liver, and some depression of leukocyte counts was observed at doses above the LD10. Previous work has shown that dogs given a lethal dose of formycin died with evidence of severe hepatotoxicity, including jaundice and increases in SGOT and SGPT; some depression of leukocyte counts was also noted. Nonlethal doses of formycin caused transient depression of leukocyte counts without evidence of liver damage (22).

When deamination of formycin is prevented by prior administration of the adenosine deaminase inhibitor dCF, the toxicity of formycin to mice is potentiated about twofold; ie, LD10 and LD50 values become 10 and 19 mg/kg/day (qd × 5 schedule), respectively. This increased toxicity is presumably due to the greater extent of analog nucleotide synthesis in susceptible organs such as the liver when the combination is used in comparison to that which occurs when formycin is given alone.

It is possible that if the dCF plus formycin combination is administered weekly rather than on 5 successive days as employed in the present studies, some of the toxic manifestations will be decreased, since such results have been observed in studies with a combination of dCF plus 8-azaadenosine (34). Also, it is conceivable that the use of a less potent inhibitor of ADA such as erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) might show biologically different effects from those seen with dCF (28,35). For example, through the use of appropriate doses of EHNA, whole-body ADA inactivation will be restricted to a few hours as compared to the long-lasting inhibition of this enzyme by dCF (28,36–38). This could then allow for the degradation of formycin nucleotides and their elimination from the liver, with decreased hepatotoxicity.

The biochemical studies reported here may help explain the hepatotoxicity seen with formycin. Not only do formycin nucleotides accumulate in the livers of treated mice, but also, formycin-5′-triphosphate can completely replace the ATP of isolated rat hepatocytes when these cells are incubated in vitro with the combination of formycin plus dCF. Since ATP is important for many cellular processes, especially those concerned with energy metabolism, it is possible that replacement of ATP by formycin-5′-triphosphate inhibits vital processes, which results in hepatotoxicity. Additional studies will be needed to evaluate this question further.

Formycin has been reported to be cytotoxic to several experimental neoplasms including Yoshida rat sarcoma, Ehrlich ascites cells, and HeLa cells (4,6,21–23). The growth inhibition of Ehrlich ascites cells by formycin has been confirmed by Caldwell et al (2). These workers also noted that a subline of Ehrlich cells, resistant to 6-methylmercaptopurine ribonucleoside (an adenosine analog) as a consequence of adenosine kinase deficiency, was cross-resistant to formycin.

In combination with dCF, formycin has been tested against human colon and pancreatic tumor cells grown in culture (30,31). In comparison with the results obtained for formycin alone, pretreatment of cells with dCF caused significant potentiation of the cytotoxic effects of formycin. In addition, the antitumor effectiveness of formycin against human pancreatic tumor xenografts in antithymocyte serum-immunosuppressed mice is potentiated when dCF is administered 1 hour prior to formycin (31).

Based on the antitumor activity seen in these various studies, we are investigating the inhibitory effects of formycin alone and in combination with dCF against human tumor xenografts hosted by athymic mice. The toxicology data presented in this paper are necessary for the selection of appropriate drug doses and schedules for such experiments.

REFERENCES

1. Hori M, Ito E, Takita T, et al. A new antibiotic, formycin. J Antibiot (Tokyo) 27:96–99, 1964.
2. Caldwell IC, Henderson JF, and Paterson ARP. Metabolism of formycin by the Ehrlich ascites carcinoma and a resistant subline. Proc Am Assoc Cancer Res 7:11, 1966.
3. Henderson JF, Paterson ARP, Caldwell IC, et al. Biochemical effects of formycin, an adenosine analog. Cancer Res 27:715–719, 1967.
4. Umezawa H, Sawa T, Fukagawa Y, et al. Studies on formycin and formycin B in cells of Ehrlich carcinoma and *E. coli*. J Antibiot (Tokyo) 20:308–316, 1967.
5. Caldwell IC, Henderson JF, and Paterson ARP. Metabolism of formycin, an adenosine analog. Can J Biochem 47:901–908, 1969.
6. Umezawa H, Sawa T, Fukagawa Y, et al. Transformation of formycin to formycin B and their biological activities. J Antibiot (Tokyo) 18:178–181, 1965.
7. Ishizuka M, Sawa T, Koyama G, et al. Metabolism of formycin and formycin B in vivo. J Antibiot (Tokyo) 21:1–4, 1968.
8. Tsukada I, Kunimoto T, Hori M, et al. Isolation and properties of the enzyme which catalyzes the oxidation of formycin B. J Antibiot (Tokyo) 22:36–38, 1969.
9. Sheen MR, Martin HF, and Parks RE, Jr. The interaction of the nucleoside analogs, formycins A and B, with xanthine oxidase and hepatic aldehyde oxidase. Mol Pharmacol 6:255–265, 1970.
10. Wong PCL, and Murray AW. 5-Phosphoribosyl pyrophosphate synthetase from Ehrlich ascites tumor cells. Biochemistry 8:1608–1614, 1969.
11. Bagnara AS, Letter AA, and Henderson JF. Multiple mechanisms of regulation of purine biosynthesis de novo in intact tumor cells. Biochim Biophys Acta 374:259–270, 1974.
12. Ikehara M, Murao K, Harada F, et al. Synthesis of formycin triphosphate and its incorporation into ribopolynucleotide by DNA-dependent RNA polymerase. Biochim Biophys Acta 155:82–90, 1968.
13. ———. Protein synthesis directed by polynucleotide containing formycin. Biochim Biophys Acta 174:696–703, 1969.
14. Ward DC, Fuller W, and Reich E. Stereochemical analysis of the specificity of pancreatic RNase with polyformycin as substrate: differentiation of the transphosphorylation and hydrolysis reactions. Proc Natl Acad Sci USA 62:581–588, 1969.
15. Ward DC, Cerami A, Reich E, et al. Biochemical studies of the nucleoside analog, formycin. J Biol Chem 244:3243–3250, 1969.
16. Ward DC, and Reich E. Conformational properties of polyformycin: a polyribonucleotide with individual residues in the syn conformation. Proc Natl Acad Sci USA 61:1494–1501, 1968.
17. Ward DC, Reich E, and Stryer L. Fluorescence studies of nucleotides and polynucleotides. I. Formycin, 2-aminopurine riboside, 2,6-diaminopurine riboside, and their derivatives. J Biol Chem 244:1228–1237, 1969.
18. Darlix JL, Fromageot P, and Reich E. Analysis of transcription in vitro using purine nucleoside analogs. Biochemistry 10:1525–1531, 1971.
19. Takeuchi T, Iwanaga J, Aoyagi T, et al. Antiviral effect of formycin and formycin B. J Antibiot (Tokyo) 19:286–287, 1966.
20. Ishida N, Homma M, Kumagai K, et al. Studies on the antiviral activity of formycin. J Antibiot (Tokyo) 20:49–52, 1967.
21. Ishizuka M, Ito E, Takita T, et al. Antitumor activities of formycin and labilomycin. J Antibiot (Tokyo) 17:124–126, 1964.
22. Ishizuka M, Sawa T, Hori S, et al. Biological studies on formycin and formycin B. J Antibiot (Tokyo) 21:5–12, 1968.
23. Kunimoto T, Wakashiro T, Okamura I, et al. Structural requirements for formycin activity. J Antibiot (Tokyo) 21:468–470, 1968.
24. Odaka T, Takizawa K, Yamaura K, et al. Immunosuppressive effects of formycins. Jpn J Exp Med 39:327–329, 1969.
25. Willemot J, Martineau R, DesRosiers C, et al. Inhibition of purine nucleoside phosphorylase and mitogen-stimulated transformation in immunocompetent spleen cells by formycin B. Life Sci 25:1215–1222, 1979.
26. Sheen MR, Kim BK, and Parks RE, Jr. Purine nucleoside phosphorylase from human erythrocytes. III. Inhibition by the inosine analog formycin B of the isolated enzyme and of nucleoside metabolism in intact erythrocytes and sarcoma 180 cells. Mol Pharmacol 4:293–299, 1968.
27. Muller WEG, Rohde HJ, Steffen R, et al. Influence of formycin B on polyadenosine diphosphoribose synthesis in vitro and in vivo. Cancer Res 35:3673–3681, 1975.
28. Agarwal RP, Spector T, and Parks RE, Jr. Tight-binding inhibitors-IV. Inhibition of adenosine deaminase by various inhibitors. Biochem Pharmacol 26:359–367, 1977.
29. Agarwal RP, Cha S, Crabtree GW, et al. Coformycin and deoxycoformycin: tight-binding inhibitors of adenosine deaminase. *In* Chemistry and Biology of Nucleosides and Nucleotides (Harmon RE, Robins RK, and Townsend LB, eds). New York, Academic Press, 1978, pp 159–197.
30. Crabtree GW, Dexter DL, Spremulli EN, et al. Biochemical and biological correlates of adenosine analog activity against cultured human colon cancer cells. Proc Am Assoc Cancer Res and ASCO 22:250, 1981.
31. Chu MY, Bogden AE, Weimann MC, et al. Growth and innovative chemotherapeutic approaches to human pancreatic carcinoma. Proc Am Assoc Cancer Res and ASCO 22:231, 1981.
32. Seglen PO. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. Exp Cell Res 82:391–398, 1973.
33. Crabtree GW, Nelson JA, and Parks RE, Jr. Failure of 6-thioGMP to inhibit guanylate kinase in intact cells. Biochem Pharmacol 26:1577–1584, 1977.
34. Spremulli EN, Crabtree GW, Dexter DL, et al. Biochemical pharmacology and toxicology of 8-azaadenosine alone and in combination with 2′-deoxycoformycin (Pentostatin). Biochem Pharmacol. 31:2415–2421, 1982.
35. Schaeffer HJ, and Schwender CF. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. J Med Chem 17:6–8, 1974.
36. Chassin MM, Adamson RH, Zaharevitz DW, et al. Enzyme inhibition titration assay for 2′-deoxycoformycin and its application to the study of the relationship between drug concentration and tissue adenosine deaminase in dogs and rats. Biochem Pharmacol 29:1849–1855, 1979.

37. Rogler-Brown T, Agarwal RP, and Parks RE, Jr. Tight-binding inhibitors. VI. The interaction of deoxycoformycin and adenosine deaminase in intact human erythrocytes and Sarcoma 180 cells. Biochem Pharmacol 27:2289–2296, 1978.
38. Agarwal RP. Deoxycoformycin toxicity in mice after long-term treatment. Cancer Chemother Pharmacol 5:83–87, 1980.

Antitumor Activity of Tetrahydro-2-furanyl- and Tetrahydro-2-pyranyl-glucans Obtained by Chemical Modification of (1→3)-β-D-Glucan From *Alcaligenes faecalis* var. *myxogenes* IFO 13140 and Its Lower Molecular Weight Glucans[1,2]

Takuma Sasaki,[3,*] Noriko Abiko,[3] Hiroyuki Uchida,[3] Motohiro Tanaka,[3] Kazuo Nitta,[3] Yoshio Yoshioka,[4] and Hiroaki Nomura[4]

The well-defined (1→3)-β-D-glucan with DP (degree of polymerization) 540 and the various chain-length (1→3)-β-D-glucans prepared by its hydrolysis were chemically modified to furanyl- and pyranyl-derivatives. As a primary screening, the effect of 20 derivatives thus obtained was tested on Sarcoma 180 solid tumor transplanted sc into allogeneic ICR mice; the dose was 10 mg/kg ip for 10 days. Eleven derivatives that showed a strong antitumor effect on Sarcoma 180 were also tested on Meth-A solid tumor in syngeneic mice. Of the derivatives tested, five furanyl-derivatives (F-4, F-6, F-7, F-10, and F-11) and one pyranyl-derivative (P-5) had potent antitumor effects on this syngeneic tumor system at ip doses of 10 or 30 mg/kg for 10 days. By oral administration, only two derivatives (F-10 and P-3) showed moderate antitumor activity against Sarcoma 180, without complete tumor regression. The mechanism of action of these derivatives was considered to be host-mediated and similar to that of the original glucan, because of a lack of effect in vitro. [Cancer Treat Rep 67:275–280, 1983]

A large number of published reports deal with the tumor regression obtained by use of polysaccharide (1). With few exceptions (2), however, the antitumor effect of polysaccharide is not obtained in a syngeneic tumor system but is obtained almost invariably in an allogeneic tumor system (3). Previously we found that the well-defined (1→3)-β-D-glucan from *Alcaligenes faecalis* var. *myxogenes* and its water-soluble derivative, carboxymethylglucan, strongly inhibit the growth of Sarcoma 180 solid tumor in ICR mice (4,5). However, these glucans also show a lack of antitumor activity against a syngeneic tumor system such as Meth-A solid tumor in BALB/c mice (T. Sasaki and N. Abiko, unpublished results). We therefore attempted to obtain a derivative that showed antitumor activity in a syngeneic system. Yamamoto (6) reported that furanyl- and pyranyl-derivatives obtained by chemical modification of secalonic acid D, an antitumor antibiotic from culture filtrates of *Penicillium oxalicum*, have more potent antitumor activity and lower toxicity than the parent compound and furthermore carry some immunopotentiating capacity. This prompted us to examine whether tetrahydrofuranylization or tetrahydropyranylization of the D-glucan contributes to antitumor activity.

The studies reported here examine the antitumor effect of furanyl- and pyranyl-glucans against allogeneic Sarcoma 180 and syngeneic Meth-A solid tumors in mice.

MATERIALS AND METHODS

Glucans

Lower molecular weight glucans were prepared from (1→3)-β-D-glucan [DP (degree of polymerization) 540], which is obtained from the culture filtrate of *Alcaligenes faecalis* var. *myxogenes* IFO 13140, by hydrolysis with 90% formic acid (85°C for 35 minutes) according to our method reported previously (4). General prop-

[1]Received Aug 5, 1982; revised Nov 17, 1982; accepted Dec 8, 1982.

[2]Supported in part by grants-in-aid for Cancer Research from the Ministry of Health and Welfare and the Ministry of Education, Science and Culture, Japan.

[3]Chemotherapy Division, National Cancer Center Research Institute, Tokyo, Japan.

[4]Central Research Division, Takeda Chemical Industries, Ltd, Osaka, Japan.

[*]*Reprint requests to:* Dr. Takuma Sasaki, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan.

erties of these lower molecular weight glucans were similar to those of the native glucan (DP 540), and gel filtration patterns of these preparations on a Sephadex G-200 column gave each symmetric peak, which indicated that they had a normal distribution with regard to the DP. The DPs of the respective glucans were determined by the method of Manners et al (7). PS-K, a glucan from *Coriolus versicolor* (FR.) QUÉL. of polyporaceae, was kindly supplied by Dr. S. Tsukagoshi of the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research.

Tetrahydrofuranylization and Tetrahydropyranylization

The content of tetrahydrofuranyl or tetrahydropyranyl groups in the glucan depends on the conditions used for modification. These derivatives were synthesized in the following typical ways.

Tetrahydro-2-furanyl Ether of (1→3)-β-D-glucans

2,3-Dihydrofuran (3.9 g, 55.7 mmols) and a small amount of *p*-toluenesulfonic acid (TSA) monohydrate (0.30 g) were added to a solution of the glucan (2.59 g, DP 47) in DMSO (75.6 ml), and the mixture was stirred for 1.5 hours at room temperature. The reaction mixture was diluted with water (200 ml) and neutralized; this was placed in a semipermeable membrane tube and dialyzed with running water for 2 days at room temperature. Lyophilization of the resulting mixture gave the desired product (F-9) as a colorless powder (yield, 5.0 g).

Nuclear magnetic resonance (60 Mc, d^6-DMSO) δ : 1.2–2.4 (broad signals, C'_3 and C'_4-H_2 of tetrahydro-2-furanyl group), 3.0–4.3 (broad, C'_5-H_2 of tetrahydro-2-furanyl group, C_2-H, C_3-H, C_4-H, C_5-H, and C_6-H_2 of 1,3-β-D-glucan moiety), 4.3–6.3 (broad, C'_2-H of tetrahydro-2-furanyl group, C_1-H and hydroxyl protons at C_2-, C_4-, and C_6-positions of 1,3-β-D-glucan).

The degree of substitution (DS; the average number of tetrahydro-2-furanyl groups substituted per anhydroglucose unit), estimated by the ratio of the nuclear magnetic resonance signal intensities due to the tetrahydro-2-furanyl protons to those due to the protons attached to the (1→3)-β-D-glucan, was in fair agreement with that calculated from the analytic values. In a similar manner, a variety of tetrahydro-2-furanyl derivatives were synthesized under the reaction conditions listed in table 1. The yields and analytic data of the products are also shown in table 1.

Tetrahydro-2-pyranyl Ether of (1→3)-β-D-glucans

The mixed solution of (1→3)-β-D-glucan (2.59 g, DP 47), 2,3-dihydro-4*H*-pyran (8.06 g, 96 mmols), and a small amount of TSA monohydrate (0.3 g, 1.6 mmols) was stirred for 3 hours at 40°C. The reaction mixture, after centrifugation to remove a small amount of precipitate, was dialyzed thoroughly to give the desired product (P-8) as a colorless powder (yield, 3.5 g). A variety of tetrahydro-2-pyranyl ethers of (1→3)-β-D-glucans were synthesized in a manner similar to that described above. The reaction conditions, yields, and analytic data are listed in table 2.

Mice

Female ICR mice, weighing about 23 g, were obtained from CLEA Japan, Inc. Female BALB/c mice that weighed about 20 g were purchased from Charles River Co, Inc, Japan.

Tumors

Two different transplantable tumors were used to evaluate possible antitumor effects of the derivatives of the glucans. Sarcoma 180, initially obtained from the Memorial Sloan-Kettering Cancer Center, New York, NY, has been maintained in the National Cancer Center Research Institute in an ascites form. Meth-A ascites tumor cells, originating from a sarcoma induced with 3-methylcholanthrene in a BALB/c mouse (8), were donated by the Sloan-Kettering Institute, and have also been maintained in the National Cancer Center Research Institute in BALB/c mice by serial ip passage. Unless otherwise stated, a volume of 0.05 ml (6×10^6 cells of Sarcoma 180 and 10^4 cells of Meth-A) of the 7-day-old ascites tumors was transplanted sc into the right groin of the mice. We have never observed any spontaneous regression of these tumors.

Assay of Antitumor Activity

Test samples at a suitable concentration in distilled water were injected into mice daily (0.1 ml, ip or by intubation), starting 24 hours after tumor transplantation. Mice were kept under observation for 5 weeks and then killed for final evaluation of the effect of treatment on tumor growth.

Inhibition rates were calculated by the following formula:

$$\text{inhibition rate (\%)} = [(A - B)/A] \times 100,$$

where A is the average tumor weight of the control group and B is that of the treated group.

In Vitro Cytotoxicity Testing

The ascites tumor cells of Sarcoma 180 (10^5 cells) or Meth-A (10^5 cells) were incubated in a CO_2 gas incubator at 37°C for 24 hours in 1 ml of medium containing various concentrations of the derivatives dissolved in

TABLE 1.—Synthesis and properties of tetrahydro-2-furanyl-(1►3)-β-D-glucans

Sample No.	PS-OH* DP	PS-OH* g	DMSO (ml)	Furan† (g)	TSA (g)	Reaction‡ (hrs)	Yield (g)	Formula§	DS
F-1	540	3.89	113	3.02	0.46	1.5	7.5	$C_6H_{7.6}O_5(C_4H_7O)_{2.40}$	2.4
F-2	540	2.00	60	1.56	0.38	3.0	2.8	$C_6H_{8.7}O(C_4H_7O)_{1.27}$	1.3
F-3	97	1.30	37.8	1.68	0.15	1.5	2.0	$C_6H_{8.5}O_5(C_4H_7O)_{1.5}$	1.5
F-4	97	1.30	37.8	0.84	0.15	1.5	1.3	$C_6H_{9.4}O_5(C_4H_7O)_{0.65}$	0.6
F-5	82	1.30	37.8	1.00	0.15	1.5	1.7	$C_6H_9O_5(C_4H_7O)_{1.02}$	1.0
F-6	49	1.30	37.8	1.68	0.15	1.5	2.0	$C_6H_{8.3}O_5(C_4H_7O)_{1.7}$	1.7
F-7	49	1.30	37.8	1.01	0.15	2.0	1.7	$C_6H_{9.2}O_5(C_4H_7O)_{0.8}$	0.8
F-8	49	1.30	37.8	0.50	0.15	1.5	1.5	$C_6H_{9.4}O_5(C_4H_7O)_{0.62}$	0.6
F-9	47	2.59	75.6	3.90	0.30	5.0	5.0	$C_6H_{7.6}O_5(C_4H_7O)_{2.4}$	2.4
F-10	14	1.30	37.8	1.00	0.15	1.8	1.8	$C_6H_{8.9}O_5(C_4H_7O)_{1.06}$	1.1
F-11	11	1.30	37.8	2.10	0.46	1.8	1.8	$C_6H_{8.1}O_5(C_4H_7O)_{1.9}$	1.9
F-12	9	3.24	94	5.70	0.34	5.3	5.3	$C_6H_{7.5}O_5(C_4H_7O)_{2.46}$	2.5

*(1►3)-β-D-Glucan.
†2,3-Dihydro-furan.
‡Reactions were performed at 18°C.
§Average formula based on the analytic data as an anhydroglucose unit of the product.

0.6% DMSO. In the periods of exposure tested, the volumes of DMSO used for dissolving the water-insoluble derivatives had no toxicity on either Sarcoma 180 or Meth-A tumor cells. Viability of tumor cells, estimated by staining with 0.17% trypan blue, was then compared with that of control cells incubated in the same medium without derivatives. RPMI-1640 medium (Meth-A) and Eagle's minimum essential medium (Sarcoma 180) with 10% fetal calf serum were used for suspension cultures.

RESULTS

Effect of Furanyl- and Pyranyl-glucans With Various Chain-Lengths and Substitutions on Sarcoma 180 in ICR Mice

As a primary screening, the effect of furanyl-glucans was tested on Sarcoma 180 transplanted sc into mice. As shown in table 3, the furanyl-glucans showed various antitumor effects when given in a daily ip dose of 10 mg/kg for 10 days from the day after tumor transplantation. There was no detectable toxicity to mice with this dose, the LD50 in mice being > 1500 mg/kg ip. F-1 and F-3 showed only mild inhibition rates (25.3% and 30.4%, respectively), with complete regression in one of six mice tested at the 10-mg/kg dose. Administration of F-2 led to a more substantial inhibition rate (65.6%), without complete tumor regression. F-4, F-5, F-6, F-7, F-8, and F-9 resulted in tumor inhibition rates of > 80% and caused complete disappearance of the tumor in many animals. F-10 demonstrated relatively high antitumor activity (inhibition rate of 73.2%) but did not cause tumor regression. Interestingly, F-11 showed a somewhat low inhibition rate of 69.0%, with complete regression in four of six mice tested at the smaller dose of 10 mg/kg; however, at a larger dose (30 mg/kg), this derivative had a high tumor inhibition rate

TABLE 2.—Synthesis and properties of tetrahydro-2-pyranyl-(1►3)-β-D-glucans

Sample No.	PS-OH* DP	PS-OH* g	DMSO (ml)	Pyran† (g)	TSA (g)	Reaction Temperature (°C)	Reaction Hrs	Yield (g)	Formula‡	DS
P-1	540	6.40	150	3.50	0.4	40	3.0	6.7	$C_6H_{9.9}O_5(C_5H_9O)_{0.09}$	0.09
P-2	125	6.40	150	3.50	0.4	90	4.0	6.1	$C_6H_{9.9}O_5(C_5H_9O)_{0.08}$	0.08
P-3	125	6.40	150	3.50	0.4	90	4.0	6.0	$C_6H_{9.9}O_5(C_5H_9O)_{0.05}$	0.05
P-4	97	1.30	37.8	2.00	0.15	18	1.5	1.8	$C_6H_{9.2}O_5(C_5H_9O)_{0.79}$	0.79
P-5	85	5.18	152	0.70	0.6	18	1.5	5.0	$C_6H_{9.9}O_5(C_5H_9O)_{0.06}$	0.06
P-6	82	1.30	37.8	2.50	0.15	18	2.0	1.5	$C_6H_{9.4}O_5(C_5H_9O)_{0.62}$	0.62
P-7	49	1.30	37.8	2.00	0.15	18	2.0	1.7	$C_6H_{9.4}O_5(C_5H_9O)_{0.65}$	0.65
P-8	47	2.59	76.0	8.06	0.3	40	3.0	3.5	$C_6H_{8.3}O_5(C_5H_9O)_{1.70}$	1.70

*(1►3)-β-D-Glucan.
†2,3-Dihydro-4*H*-pyran.
‡Average formula based on the analytic data as an anhydroglucose unit of the product.

TABLE 3.—Effect of furanyl- and pyranyl-glucans against Sarcoma 180 at an ip dose of 10 mg/kg for 10 days

Sample No.	Tumor weight (g) (treated/control mice)*	Tumor inhibition rate (%)	Complete regression†
F-1	5.3 ± 3.4/7.1 ± 2.5	25.3	1/6
F-2	2.3 ± 1.2/6.7 ± 4.4	65.6	0/6
F-3	3.2 ± 2.0/4.6 ± 4.3	30.4	1/6
F-4	0.5 ± 0.4/4.6 ± 4.3	89.1	2/5
F-5	0.9 ± 0.7/5.8 ± 3.6	84.4	3/6
F-6	0.6 ± 0.8/4.6 ± 4.3	86.9	3/5
F-7	0.9 ± 1.8/4.6 ± 4.3	80.4	4/6
F-8	0.4 ± 0.8/4.6 ± 4.3	91.3	4/5
F-9	0.6 ± 0.8/7.1 ± 2.5	91.5	3/5
F-10	1.9 ± 1.0/7.1 ± 2.5	73.2	0/6
F-11	2.2 ± 4.4/7.1 ± 2.5	69.0	4/6
	0.3 ± 0.6/7.1 ± 2.5‡	95.7	3/5
F-12	3.9 ± 2.0/7.1 ± 2.5	45.0	0/6
P-1	0.1 ± 0.1/6.7 ± 4.4	98.5	5/6
P-2	3.4 ± 3.1/6.7 ± 4.4	49.2	1/6
P-3	0 /6.7 ± 4.4	100	5/5
P-4	2.7 ± 2.5/4.6 ± 4.3	41.3	0/5
P-5	0 /5.3 ± 4.4	100	5/5
P-6	3.7 ± 3.0/5.8 ± 3.6	36.2	0/6
P-7	3.6 ± 2.2/4.6 ± 4.3	21.1	0/6
P-8	2.7 ± 1.8/4.9 ± 1.9	44.8	0/6

*Values = mean ± SD.
†Values = No. of tumor-free mice/No. tested.
‡At a dose of 30 mg/kg for 10 days.

of 95.7% and caused regression in three of five mice tested. F-12, which has the lowest DP, showed a moderate inhibition rate of 45.0%, without complete tumor regression.

Table 3 also shows the antitumor effect of the pyranyl-glucans. P-1, P-3, and P-5 had a strong antitumor activity and caused complete tumor regression in many animals at an ip dose of 10 mg/kg for 10 days. However, none of the pyranyl-glucans showed antitumor effects at a dose of 1 mg/kg. P-2, P-4, and P-8 showed a moderate inhibition rate, and P-6 and P-7 a low inhibition rate at the 10-mg/kg dose.

Effect of Furanyl- and Pyranyl-glucans on Meth-A Solid Tumor in BALB/c Mice

Furanyl- and pyranyl-glucans that showed a potent antitumor activity against Sarcoma 180 were also tested on Meth-A tumor cells transplanted sc into syngeneic mice. Results are summarized in table 4. The inhibition rate of the native (1→3)-β-D-glucan with DP 540, when tested at a daily dose of 10 mg/kg, was 43.3%; at a daily dose of 30 mg/kg, the inhibition rate was 13.3%. A daily dose of 10 mg/kg of the furanyl-glucans F-6, F-8, and F-10 resulted in mild inhibition rates (33.3%, 36.6%, and 23.3%, respectively). However, F-6 and F-10 showed a potent antitumor effect at a daily dose of 30 mg/kg (inhibition rates of 83.3% and 76.6%, with complete tumor regression in one of five and two of five mice tested, respectively). F-4 and F-7 had a high antitumor activity at the 10-mg/kg dose, both with inhibition rates of 73.3% and many complete tumor regressions. F-11 exhibited a high inhibition rate of 93.3%, with complete tumor regression in three of six mice tested at the 30-mg/kg dose for 10 days. In contrast, F-5 at the 10-mg/kg dose had a completely different effect, resulting in a slight increase in tumor weight (inhibition rate, –23.3%).

Pyranyl-glucans listed in table 3 had no antitumor effect (data not shown), except for P-5 which showed an inhibition rate of 86.6%, with complete tumor regression in three of six mice tested (table 4).

Effect of Furanyl- and Pyranyl-glucans by Oral Administration

The effects of five orally administered furanyl-glucans and two orally administered pyranyl-glucans against Sarcoma 180 were investigated. The results of bioassay are shown in table 5. The original (1→3)-β-D-glucan and PS-K, a glucan from *Coriolus versicolor* (FR.) QUÉR. of polyporaceae (*Basidiomycetes*) which has been reported to exhibit antitumor activity against Sarcoma 180 after oral administration (9), had no antitumor activity by oral administration. Among furanyl- and pyranyl-derivatives, P-3 indicated a moderate inhibition

TABLE 4.—Effect of furanyl- and pyranyl-glucans on Meth-A tumor in syngeneic BALB/c mice

Sample No.	Dose (mg/kg/day, × 10 days)	Tumor weight (g)*	Tumor inhibition rate (%)	Complete regression†
F-4	10	0.8 ± 1.0	73.3	3/6
F-5	10	3.7 ± 3.5	−23.3	0/6
F-6	10	2.0 ± 1.8	33.3	2/6
	30	0.5 ± 0.4	83.3	1/5
F-7	10	0.8 ± 1.3	73.3	4/6
F-8	10	1.9 ± 1.7	36.6	1/6
F-10	10	2.3 ± 2.7	23.3	2/6
	30	0.7 ± 1.1	76.6	2/5
F-11	30	0.2 ± 0.3	93.3	3/6
P-5	10	0.4 ± 0.8	86.6	3/6
(1→3)-β-D-glucan	10	1.7 ± 2.9	43.3	1/5
	30	2.6 ± 2.0	13.3	0/6
Control		3.0 ± 3.1		0/7

*Values = mean ± SD.
†Values = No. of tumor-free mice/No. tested.

rate of 65.6% at a dose of 1000 mg/kg for 10 days, and F-10 also showed a moderate inhibition rate of 67.7% at a dose of 500 mg/kg. However, neither derivative caused tumor regression.

Direct Cytocidal Action of the Derivatives on Tumor Cells

To examine whether the derivatives had a direct cytocidal effect on tumor cells, ascites cells of Sarcoma 180 or Meth-A were cultured in a medium containing concentrations of 0.1–0.5 mg of each derivative/ml. The viability of the respective tumor cells was examined by trypan blue staining and was found to be > 95%, which did not differ from that of the cells cultured without addition of the derivatives. It is clear that these derivatives had no direct cytotoxicity against the tumor cells used.

DISCUSSION

The present investigations concern the effects of glucan derivatives. The purpose of this study was to focus on the relatively few compounds worthy of more detailed examination. Particular attention was given to the question of whether the glucan derivatives might exert their inhibitory effects on syngeneic tumor systems. We tested the efficacy of the glucan derivatives as therapeutic agents in two experimental tumor systems under well-defined experimental conditions.

As a primary screening, Sarcoma 180 was selected for evaluation of the antitumor activity of the deriva-

TABLE 5.—Effect of furanyl- and pyranyl-glucans on Sarcoma 180 by oral administration

Sample No.	Dose (mg/kg/day × 10 days)	Tumor weight (g) (treated/control mice)*	Tumor inhibition rate (%)	Complete regression†
F-6	500	2.8 ± 1.7/4.5 ± 1.2	37.7	0/6
F-8	500	3.1 ± 1.3/4.5 ± 1.2	31.1	0/6
F-10	500	1.0 ± 1.0/3.1 ± 2.0	67.7	0/6
F-11	500	6.4 ± 2.6/7.4 ± 3.3	13.5	0/6
F-12	1000	7.3 ± 1.8/7.4 ± 3.3	1.3	0/6
P-1	1000	4.5 ± 1.6/6.7 ± 4.4	32.8	0/6
P-3	1000	2.3 ± 0.4/6.7 ± 4.4	65.6	0/6
(1→3)-β-D-glucan	1000	5.7 ± 1.6/7.4 ± 3.3	22.9	0/6
PS-K	500	3.2 ± 2.2/4.5 ± 1.2	28.8	0/6

*Values = mean ± SD.
†Values = No. of tumor-free mice/No. tested.

tives. Our previous results show that the original (1→3)-β-D-glucans with DP > 50 have a strong antitumor activity against this tumor when injected ip at a daily dose of > 5 mg/kg for 10 days (4). In addition to DP, which plays a dominant role in the antitumor activity of polysaccharide, the present results show that the DS of furanyl groups also affects antitumor activity. It is noteworthy that oligosaccharides, which have little antitumor activity, show a strong antitumor activity after furanylization (eg, F-11). When the DP is high, a low degree of furanyl substitution was required for antitumor activity. However, when the DP is low, the antitumor activity remains high even with a high DS.

Pyranyl-derivatives P-1, P-3, and P-5 showed a potent antitumor activity, but the other pyranyl derivatives decreased or lost their activity by the introduction of pyranyl groups. For antitumor activity, the limit to the content of this group seems to be about 0.1 per glucose residue.

The inhibition rates observed with oral administration were less than the values achieved with ip injection, but those of F-10 and P-3 were clearly higher than those of the original (1→3)-β-D-glucans and PS-K. The unexpected finding that the derivative of oligosaccharide (F-11) was not active is perplexing, and the results presented here suggest that no general conclusions on structure-activity relationship can yet be made about the effect of orally administered furanyl- and pyranyl-derivatives.

The antitumor activity of polysaccharides differs among mouse strains. BALB/c mice are especially weak responders to antitumor polysaccharides. The derivatives that showed antitumor activity against Sarcoma 180 in ICR mice were further tested on Meth-A transplanted sc into BALB/c mice. It seems appropriate to use this system, which is syngeneic in the relationship of tumor to host, for preliminary detection of the glucan derivatives that will possess a significant antitumor effect in a syngeneic tumor system. Table 4 shows that there is great variation in the susceptibility of the Meth-A tumor to the curative effect of ip injection of the derivatives that exhibited marked antitumor activity against Sarcoma 180. Of the derivatives tested, the introduction of furanyl groups (F-4, F-6, F-7, F-10, and F-11) and pyranyl groups (P-5) does indeed increase the antitumor potency. No information is available on whether these derivatives have a direct antitumor effect or whether they are effective after hydrolysis in vivo to glucan. Probably these active antitumor derivatives have a direct effect because the original (1→3)-β-D-glucan and its lower molecular weight glucans have no antitumor activity against Meth-A transplanted sc into BALB/c mice. Also, 2,3-dihydro-furan and 2,3-dihydro-4*H*-pyran given alone had no antitumor activity on either Sarcoma 180 or Meth-A (data not shown).

The derivatives described above were also tested in vitro. There is a sharp contrast in their effects, depending on whether tumor growth inhibition is studied in vitro or in vivo. The derivatives did not interfere with the growth of Sarcoma 180 or Meth-A in vitro but did retard the growth of these cells in animals. Consequently, the antitumor effect of these derivatives is considered to be host-mediated, ie, by defense mechanisms of the host similar to those mediating the effects of the original D-glucan (4) and other polysaccharide preparations (10).

The derivatives reported in this paper are the first chemically modified polysaccharides to show antitumor activity in a syngeneic tumor system and by oral administration. A number of questions must be resolved before a clear statement regarding the relationships between chemical structure and biologic activity can be made. There remains the possibility that since a multiplicity of dose schedules were not tested relative to the use of each derivative, the derivatives may not have been employed under optimal circumstances in these models. Further experiments are also necessary to extend our results to other syngeneic tumor systems. This could lead to the synthesis of more potent polysaccharide derivatives in the future.

REFERENCES

1. Whistler RL, Bushway AA, Singh PP, et al. Non-cytotoxic, antitumor polysaccharides. Adv Carbohydr Chem Biochem 32:235–274, 1976.
2. Kohno M, Abe S, Yamazaki M, et al. Antitumor effect of polysaccharide TC-13 on allogeneic and syngeneic tumors in mice. Gan 73:488–494, 1982.
3. Hunter JT, Meltzer MS, Ribi E, et al. Glucan: attempts to demonstrate therapeutic activity against five syngeneic tumors in guinea pig and mice. JNCI 60:419–424, 1978.
4. Sasaki T, Abiko N, Sugino Y, et al. Dependence of chain length of antitumor activity of (1→3)-β-D-glucan from *Alcaligenes faecalis* var. *myxogenes* IFO 13140, and its acid-degraded products. Cancer Res 38:379–383, 1978.
5. Sasaki T, Abiko N, Nitta K, et al. Antitumor activity of carboxymethylglucans obtained by carboxymethylation of (1→3)-β-D-glucan from *Alcaligenes faecalis* var. *myxogenes* IFO 13140. Eur J Cancer 15:211–215, 1979.
6. Yamamoto T. Antitumor antibiotics carrying immunopotentiating capacity. *In* Host Defense Against Cancer and Its Potentiation (Mizuno D, Chihara G, Fukuoka F, et al, eds). Tokyo, University of Tokyo Press, 1975, pp 379–386.
7. Manners DJ, Masson AJ, and Sturgeon RJ. An enzymic method for the determination of the degree of polymerization of glucans. Carbohydr Res 17:109–114, 1971.
8. Old LJ, Boyse EA, Clark DA, et al. Antigeneic properties of chemically induced tumors. Ann NY Acad Sci 101:80–106, 1962.
9. Tsukagoshi S, and Ohashi F. Protein-bound polysaccharide preparation, PS-K effective against mouse Sarcoma 180 and rat ascites hepatoma AH-13 by oral use. Gan 65:557–558, 1974.
10. Nakahara W, Fukuoka F, Maeda Y, et al. The host-mediated antitumor effect of some plant polysaccharides. Gan 55:282–288, 1964.

Intermittent High-Dose Aclarubicin in Patients With Advanced Cancer: A Phase I Study With Special Reference to Cardiac Toxicity[1,2,3]

Kristian Aabo,[4,*] Svend Aage Mortensen,[5] Torben Skovsgaard,[4] and Erik Gymoese[5]

Aclarubicin was given iv every 3 weeks to 21 patients with a variety of solid tumors. Four dose levels were tested: 80, 100, 120, and 140 mg/m². The median cumulative dose was 270 mg/m² (range, 80–900). No objective responses were observed. Hematologic toxicity was dose-limiting. All patients experienced moderate to severe gastrointestinal toxicity. Five of 13 patients evaluable for cardiac function showed clinical signs and ECG changes suggestive of cardiac dysfunction. The suggested maximum dose for phase II trials is 120–140 mg/m² iv given in an intermittent 3-week schedule. [Cancer Treat Rep 67:281–282, 1983]

Aclarubicin was isolated from the culture of *Streptomyces galilaeus* by Oki et al in 1975 (1). In animal experiments, aclarubicin has demonstrated a lower cardiac toxicity than doxorubicin (2,3). Clinical evaluation of aclarubicin was started in 1977 in Japan (4,5). The purpose of the present study was to determine the maximal tolerated single iv dose and the toxicity in an intermittent 3-week schedule.

MATERIALS AND METHODS

Patients with histologically proven malignant solid tumors were eligible for the study. Other inclusion criteria were: no previous anthracycline therapy, measurable or evaluable disease, normal liver and renal functions, and no history or clinical signs of heart disease. Routine pretreatment evaluation and weekly follow-up were performed. Cardiac evaluation performed every 6 weeks included: physical examination, heart volume per square meter measured from chest x-rays (6), and serial ECGs. The absolute sum of the QRS voltages in the six standard leads was calculated, as was the change in percent from the baseline ECG. Holter monitoring with determination of heart rate during the 24-hour period was used to compare median nocturnal heart rate with the pretreatment recordings. Echocardiograms were obtained in selected cases. Aclarubicin was given iv every 3 weeks. Four dose levels were tested: 80 mg/m² (six patients), 100 mg/m² (five), 120 mg/m² (five), and 140 mg/m² (five). Response was defined according to the World Health Organization recommendations. Informed consent was given by all patients.

RESULTS

Twenty-one patients were entered in the study. The male:female ratio was 8:13 and the median age was 57 years (range, 38–72). Four patients had received no prior therapy, eight had had prior chemotherapy only, seven had received prior irradiation only, and two had had prior chemotherapy and radiotherapy. The tumor sites were: lung (epidermoid carcinoma and adenocarcinoma), seven patients; breast, three; ovary, two; kidney, two; cervix, two; head and neck, two; urinary bladder, one; mesothelioma, one; and metastatic adenocarcinoma of unknown origin, one. The median number of courses was three (range, one to nine). The median total cumulative dose was 270 mg/m² (range, 80–900), with five patients receiving > 500 mg/m².

The hematologic toxicity was dose-dependent and dose-limiting, with a median wbc count nadir of 1.7×10^9/L (range, 0.7–2.8) and a median platelet count nadir of 72×10^9/L (range, 19–137) at a dose level of 140 mg/m². The median time from drug administration to wbc count nadir was 21 days (range, 12–21) and to platelet

[1]Received Mar 30, 1982; revised Nov 24, 1982; accepted Dec 21, 1982.

[2]Presented in part at the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Belgium, Oct 1981.

[3]Aclarubicin is produced by Sanraku-Ocean Co, Ltd, Tokyo, Japan, in 20-mg vials with 100 ml of 0.9% saline as solvent, and was supplied by H. Lundbeck & Co, Copenhagen, Denmark.

[4]Department of Chemotherapy, Finsen Institute, Copenhagen, Denmark.

[5]Department of Medicine B, Rigshospitalet, Copenhagen, Denmark.

**Reprint requests to:* Kristian Aabo, MD, Department of Chemotherapy R II, Finsen Institute, 49 Strandboulevarden, DK-2100 Copenhagen, Denmark.

count nadir was 7 days (range, 7–21). Three patients developed severe leukopenia, thrombocytopenia, sepsis, and stomatitis. There was no death due to acute hematologic toxicity. Nine patients developed anemia (hemoglobin < 6 mmols/L). All patients experienced some degree of gastrointestinal toxicity, with 14 patients having severe and prolonged emesis. Five patients developed signs of hepatic toxicity, with transient increase in SGOT. Two patients experienced muscle pain and two had partial alopecia. Five of 13 patients evaluable for cardiac function (followed two or more times) showed clinical signs and ECG changes suggestive of cardiac dysfunction. All five patients were female, the median age was 54 years (range, 44–62), and the median cumulative aclarubicin dose was 480 mg/m^2 (range, 160–600). Four patients developed edema of the lower extremities or neck vein distension, three with concomitant decrease in QRS voltage (18%–24%). One of these patients (a 44-year-old female) died with overt cardiac failure with pulmonary edema (total dose, 480 mg/m^2). Four patients developed permanent ECG changes (two had sinus tachycardia, one had multiple premature ventricular contractions, and one had alteration in T waves and ST segments). None of the patients had significant increase in heart volume during the treatment. Six patients had a > 10% increase in median night heart rate (13%–49%) measured by Holter monitoring. Four of these patients did not show other signs of altered cardiac function. Postmortem histologic examination of the heart was obtained only in a 38-year-old male receiving a total dose of 240 mg/m^2 and showed increased diffuse interstitial fibrosis without evidence of coronary atherosclerosis. No objective responses were observed.

DISCUSSION

Aclarubicin has been reported to be active in solid tumors including breast, lung, ovary, cervix, and stomach carcinomas (7). In the present study, there were no objective responses among 21 patients. In other phase I studies (4,5,8,9), the response rates have also been disappointingly low, ie, only two partial remissions among 79 patients with solid tumors in these studies. Hematologic toxicity, emesis, and hepatic toxicity in our study was equivalent to that observed by others (4,5,9,10).

In animal experiments, the cumulative cardiac toxicity of aclarubicin has been reported to be reduced compared to conventional anthracyclines (2,3). However, this critical toxicity has been poorly evaluated in man. Transient ECG changes, most frequently flattening or inversion of T waves or sinus tachycardia, have been reported (7,11). In a recent study (9), permanent ECG changes (complete heart block) were observed at fairly low cumulative doses using the same monitoring as used in the present study. According to Minow et al (12), a ≥ 30% decrease in QRS voltage is highly correlated with the development of congestive heart failure in patients treated with doxorubicin. Three patients developed decrease in voltage (18%–24%), with accompanying signs of right-sided stasis. However, these figures are below the minimum value described by Minow et al, and signs of right-sided stasis may occur for many reasons in patients with advanced cancer. Additionally, tachycardia in this patient population might be nonspecific. Further cardiac evaluation including ejection fraction in patients receiving aclarubicin is thus necessary before final conclusions concerning aclarubicin cardiac toxicity can be made. However, at present it seems that aclarubicin may be more cardiotoxic than initially believed.

REFERENCES

1. Oki T, Matsuzawa Y, Yoshimoto A, et al. New antitumor antibiotics, aclacinomycin A and B. J Antibiot 28:830–834, 1975.
2. Hori S, Shirai M, Hirano S, et al. Antitumor activity of new anthracycline antibiotics, aclacinomycin A and its analogs, and their toxicity. Gan 68:685–690, 1977.
3. Wakabayashi T, Oki T, Tone H, et al. A comparative electron microscopic study of aclacinomycin and adriamycin cardiotoxicities in rabbits and hamsters. J Electron Microsc 29:106–118, 1980.
4. Furue H, Komita T, Nakao I, et al. Clinical experiences with aclacinomycin A. Recent Results Cancer Res 63:241–264, 1978.
5. Ogawa M, Inagaki J, Horikoshi N, et al. Clinical study of aclacinomycin A. Cancer Treat Rep 63:931–934, 1979.
6. Jonsell S. A method for the determination of heart size by teleroentgenography (a heart volume index). Acta Radiol 20:325–345, 1939.
7. Oka S. Review of clinical studies on aclacinomycin A—phase I and preliminary phase II evaluation of ACM. Sci Rep Res Inst Tohoku Univ [Med] 25:37–49, 1978.
8. Sakano T, Okazaki N, Ise T, et al. Phase I study of aclacinomycin A. Jap J Clin Oncol 8:49–53, 1978.
9. Van Echo DA, Whitacre MY, Aisner J, et al. Phase I trial of aclacinomycin A. Cancer Treat Rep 66:1127–1132, 1982.
10. Casper ES, Gralla RJ, and Young CW. Clinical phase I study of aclacinomycin A by evaluation of an intermittent intravenous administration schedule. Cancer Res 41:2417–2420, 1981.
11. Yamada K, Nakamura T, Tsuruo T, et al. A phase II study of aclacinomycin A in acute leukemia in adults. Cancer Treat Rev 7:177–182, 1980.
12. Minow RA, Benjamin RS, Lee ET, et al. QRS voltage change with adriamycin administration. Cancer Treat Rep 62:931–934, 1978.

Dialyzability of Methyl-GAG[1]

Barbara Jensen,[2] Richard D. Williams,[2,*] Takao Ohnuma,[3] and John Roboz[3]

Methyl-GAG at low doses was administered to an anephric male patient with metastatic hypernephroma, who required hemodialysis. No toxicity was observed. Measurement of serum levels of methyl-GAG showed that the drug is dialyzable. Thus, when methyl-GAG is considered for patients who are anephric and are being dialyzed, full doses are allowable. In addition, dialysis should be effective in rapidly eliminating the drug in patients who develop toxic effects. [Cancer Treat Rep 67:283–284, 1983]

Methyl-GAG is a chemotherapeutic agent that may produce cytotoxic effects by inhibition of polyamine synthesis. This agent is presently being studied in clinical trials for a variety of malignancies, including tumors of the central nervous system, solid tumors, leukemias, and lymphomas. In a phase I–II study (1) of patients with advanced renal cell carcinoma, four of 25 patients (16%) responded to methyl-GAG therapy. Another recent report (2) also showed responses to methyl-GAG in three of 14 previously untreated patients (21%) with advanced renal cell carcinoma.

The pharmacokinetics (3) of methyl-GAG in patients with normal renal function show a biphasic urinary excretion of unmetabolized drug, with one-quarter of the dose excreted in the first 24 hours and a slow secondary excretion phase. Animal studies have shown a marked increase in toxicity with small changes in dose (4).

We administered methyl-GAG to an anephric man with metastatic renal cell carcinoma, who required hemodialysis. Because of the known renal excretion of the drug and its low therapeutic index, we used low doses and we measured serum and dialysate levels.

CASE REPORT

A 47-year-old white male with hematuria had a left nephrectomy for renal cell carcinoma in 1975. He developed recurrent gross painless hematuria in 1978 and a renal cell carcinoma was removed from his right kidney by partial nephrectomy. In September 1979, the patient again presented with gross hematuria, and results of urinary cytologic examination were positive. At that time he also had back pain, and a solitary osteolytic lesion at L_5 was found on bone scan. The remainder of the right kidney was infarcted by percutaneous transarterial embolization followed in 1 week by nephrectomy. A partial L_5 laminectomy was done. Histologic examination revealed metastatic renal cell carcinoma; palliative radiation therapy was therefore given to spinal level L_5. The patient continued to have repeated hemodialysis. Subsequently, an anterior chest wall mass developed, the size of which progressively increased. In July 1980, methyl-GAG therapy was begun, via low but escalating doses every 2 weeks. During the treatment there was no objective evidence of toxicity, although at the highest dose used there was subjective oral mucositis. No clinical response was evident and the patient died with widespread metastatic renal cell carcinoma in October 1980.

METHODS AND RESULTS

Methyl-GAG was given by slow iv infusion over 20–30 minutes in low doses (initially 350 mg/m^2) and was increased by 100 mg/m^2 every 2 weeks. The highest dose used was 550 mg/m^2. The drug was measured by ion-pair chromatography in both serum and dialysate according to the method described by Roboz et al (5). The limit of detection in this assay in serum and ascitic and pleural fluids is 30 ng/ml. In all of the samples studied here the levels were very low; however, the serum levels declined slowly before dialysis and more

[1]Received June 22, 1982; revised Nov 26, 1982; accepted Dec 21, 1982.

[2]Departments of Hematology-Oncology (*B. Jensen*) and Urology (*R. D. Williams*), Veterans Administration Hospital, and the University of California School of Medicine, San Francisco.

[3]Department of Neoplastic Diseases, Mt. Sinai School of Medicine, New York, NY.

**Reprint requests to:* Richard D. Williams, MD, Department of Urology, M-553, University of California School of Medicine, San Francisco, CA 94143.

TABLE 1.—Serum levels before and after dialysis

Methyl-GAG dose (mg/m^2)	Day	Time after infusion (hrs)	Drug level ($\mu g/ml$)
350	1	5	0.1
	2	24	0.1
450	15	5	1.1
	15	11	0.3
	15	17	0.4
	16	24 (predialysis)	0.3
	16	30 (postdialysis)	0.1
550	29	5	0.6
	29	11	0.6
	29	17	0.5
	30	24 (predialysis)	0.4
	30	30 (postdialysis)	0.1

abruptly immediately thereafter (table 1). The dialysate levels were < 0.1 μg/ml. It is probable that therapeutic drug levels were never obtained in this case. Yet, it is likely that the patient could have tolerated more standard doses of methyl-GAG.

DISCUSSION

Studies of the pharmacokinetics of methyl-GAG have shown varying results. One study (6) showed a biphasic pattern of plasma decay with an α-half-life of 14–19 minutes and a β-half-life of 73–163 minutes. In another study (7), the initial half-life was 1.3–3.2 hours and there was a long terminal elimination of > 40 hours for most patients. In the same study, the urinary excretion continued through Day 14. T. Ohnuma showed a triphasic serum decay (α-half-life = 0.3 hours; β-half-life = 3.0 hours; γ-half-life = 100 hours), and the drug was still detectable at 1 week. This same study demonstrated biphasic urinary excretion of unmetabolized drug with a slow secondary excretion phase. A recently reported study (8) of the pharmacokinetics of methyl-GAG labeled with ^{14}C in six patients with lymphoma and normal renal and hepatic functions revealed an average terminal half-life of 4.1 hours, but the cumulative urinary excretion was only 14.5% of the total radioactive dose. Marsh et al (9) have also demonstrated the long terminal half-life of methyl-GAG. It is not known whether the prolonged half-life of the drug is due to extensive tissue binding, tubular reabsorption, or enterohepatic recirculation. The prolonged urinary excretion may, however, contribute to increased toxicity if the drug is administered on a frequent schedule, or if it is given to a patient having inadequate renal function.

We have shown that standard doses of methyl-GAG should be well-tolerated by patients having chronic renal failure, who have to undergo hemodialysis. Furthermore, if toxicity were to occur in the course of therapy, dialysis should be effective in rapidly eliminating the drug.

REFERENCES

1. TODD RF, III, GARNICK MB, CANELLOS GP, ET AL. Phase I–II trial of methyl-GAG in the treatment of patients with metastatic renal adenocarcinoma. Cancer Treat Rep 65:17–20, 1981.
2. KNIGHT WA, LIVINGSTON RB, FABIAN C, ET AL. Methyl-glyoxal bis-guanyl hydrazone in advanced renal carcinoma. Proc Am Assoc Cancer Res and ASCO 21:367, 1980.
3. HART RD, ROBOZ J, WU K, ET AL. Clinical and pharmacologic studies with weekly and biweekly methyl-glyoxal bis-guanylhydrazone. Proc Am Assoc Cancer Res and ASCO 21:181, 1980.
4. MIHICH E. Current studies with methylglyoxal bis (guanylhydrazone). Cancer Res 23:1375–1389, 1963.
5. ROBOZ J, WU KT, and HART RD. Determination of methylglyoxal-bis (guanylhydrazone) in body fluids by ion-pair chromatography. J Anal Toxicol 4:127–131, 1980.
6. KILLEN J, HOTH D, SMITH F, ET AL. Methylglyoxal-bisguanyl-hydrazone: phase II experience and clinical pharmacology. Proc Am Assoc Cancer Res and ASCO 21:368, 1980.
7. LIESMANN J, FABIAN C, STEPHENS R, ET AL. Pharmacokinetics of methyl-glyoxal bis-guanylhydrazone. Proc Am Assoc Cancer Res and ASCO 21:151, 1980.
8. ROSENBLUM MG, KEATING MJ, YAP BS, ET AL. Pharmacokinetics of (^{14}C) methylglyoxal-bis (guanylhydrazone) in patients with leukemia. Cancer Res 41:1748–1750, 1981.
9. MARSH KC, LIESMANN J, PATTON TF, ET AL. Plasma levels and urinary excretion of methyl-GAG following iv infusion in man. Cancer Treat Rep 65:253–257, 1981.

Phase II Evaluation of Anguidine in Central Nervous System Tumors: A Southwest Oncology Group Study[1,2]

J. Wendall Goodwin,[3] Richard H. Bottomley,[4] Clarence B. Vaughn,[5] Jess Frank,[6] and Reginald P. Pugh[7,*]

The role of chemotherapy in primary central nervous system tumors remains investigational. Anguidine (diacetoxyscirpenol) is a mycotoxin extracted from the fungus *Fusarium equiseti* (1,2). A temporal relationship between cerebral dysfunction and the development of abnormal EEGs with the administration of anguidine while the cerebrospinal fluid remained normal suggested that anguidine may cross the blood-brain barrier [J. W. Goodwin, unpublished data; (3)]. Based on these data, a phase II trial was undertaken.

MATERIALS AND METHODS

Between August 1978 and April 1980, 17 patients who had disease progression on higher priority protocols or standard therapy were entered into the study. Sixteen patients were evaluated for toxicity and response. There were 12 males and four females, with a median age of 44 years (range, 23–61). All patients evaluable for response had had a subtotal (13 patients) or total (three) resection of their tumors, followed by radiation therapy; all had received prior chemotherapy, generally with nitrosourea alone or in combination. Thirteen patients had grade III or IV astrocytoma, two had medulloblastoma, and one had oligodendroglioma.

Anguidine (lyophilized form) was supplied in 5-mg vials by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Poor-risk patients were defined as being $\geq$ 65 years of age, demonstrating poor tolerance to prior chemotherapy, having serum creatinine $>$ 1.0 g/dl, or having bilirubin between 3.0 and 6.0 mg/dl or liver function tests of greater than three times the institutional norm. Values in excess of the above rendered the patient ineligible. All other patients were considered good risks. Good-risk patients received 5.0 mg/m^2 and poor-risk patients received 3.5 mg/m^2 of anguidine dissolved in 500 ml of 5% dextrose in water and infused over 4 hours. A weekly schedule was chosen (4). All patients evaluable for response, except one, were good risks.

Response criteria were based on tumor size and/or neurologic examination. Neither parameter could deteriorate in patients considered to have complete response, partial response (PR), or stable disease. A complete response was defined as the complete disappearance of measurable tumor on computerized tomographic scan (or radionuclide scan) and/or return to a normal neurologic examination independent of corticosteroids. A PR was defined as a decrease in the size of tumor and/or improvement in the neurologic examination, independent of corticosteroids. Stable disease was defined as unchanged size of tumor or unchanged neurologic examination for a period of 8 weeks, independent of corticosteroids. Increasing disease was defined as increasing size of tumor or worsening of neurologic examination. Patients had to receive at least three courses of anguidine to be evaluable for response. All patients were followed until death.

RESULTS

Of 16 patients evaluated for response, two (12.5%) achieved PR (95% confidence limits, 0–28.7, Clopper-Pearson exact test), three (18.8%) had stable disease (95% confidence limits, 1–37.6%), and nine (56.3%) had increasing disease (95% confidence limits, 32–80.5%).

Toxicity was mild and consisted of myelosuppression, gastrointestinal symptoms, and central nervous system

[1]Received June 24, 1982; revised Nov 24, 1982; accepted Jan 6, 1983.
[2]Supported in part by Public Health Service grants CA-16957, CA-22416, CA-26766, CA-03392, CA-22411, CA-03195, CA-16385, CA-21116, CA-28862, CA-20319, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Wilford Hall Medical Center, San Antonio, TX.
[4]Oklahoma Medical Research Foundation, Oklahoma City.
[5]Providence Hospital, Southfield, MI.
[6]Southwest Oncology Biostatistical Office, Houston, TX.
[7]Allegheny General Hospital, Pittsburgh, PA.
Reprint requests to: Southwest Oncology Group (SWOG-7812), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

symptoms. Of the 16 evaluable patients, three had wbc counts $< 4.0 \times 10^3/mm^3$ (range, $1.9–3.2 \times 10^3$), and four had platelet counts $< 100 \times 10^3/mm^3$ (range, $39–76 \times 10^3$). Gastrointestinal toxicity consisted of mild nausea and vomiting in five patients, and three patients reported mild dizziness or hallucinatory feelings after one or more treatments.

DISCUSSION

In a review of selected chemotherapy trials by Kornblith et al (5), the median time from relapse to death after response and subsequent failure of initial therapy with radiotherapy and chemotherapy was 13 weeks, which is comparable to the 11 weeks seen in the group with continued tumor growth (increasing disease) in this study. The improvement in survival in the groups with PR and stable disease could represent biologically less aggressive tumors; however, the lack of a significant difference in time from initial diagnosis to start of anguidine and lack of response to prior chemotherapy in the partial responders in this study suggest a beneficial effect due to anguidine in this limited study with its small sample of patients.

The size of the study and the lack of histologic proof of relapse from prior therapy preclude any definite statement relative to the activity of anguidine in central nervous system tumors. Further studies with larger patient populations and strict criteria defining study eligibility and responses are suggested (6).

REFERENCES

1. Brian PW, Dawkins AW, Graves JF, et al. Phytotoxic compounds produced in *Fusarium equiseti*. J Exp Botany 12:1–12, 1961.
2. Dawkins AW, Graves JF, and Todd BK. Diacetoxyscirpenol and some related compounds. Chem Comm 1:27–28, 1965.
3. Goodwin JW, Haas CD, Fabian CJ, et al. Phase I evaluation of anguidine (diacetoxyscirpenol, NSC-141537). Cancer 42:23–26, 1978.
4. Belt RJ, Haas CD, Joseph U, et al. Phase I study of anguidine administered weekly. Cancer Treat Rep 63:1993–1995, 1979.
5. Kornblith PL, Walker MD, and Cassaday JR, Jr. Cancer: Principles and Practice of Oncology (DeVita VT, Hellman S, and Rosenberg SA, eds). Philadelphia, J. B. Lippincott, 1982.
6. Levin VA, Crafts DC, Norman DM, et al. Criteria for evaluating patients undergoing chemotherapy for malignant brain tumors. J Neurosurg 47:329–335, 1977.

Cyclophosphamide, Doxorubicin, Vincristine, and Dexamethasone in Primary Lymphoma of the Brain: A Case Report[1]

David J. Stewart,[2,3,*] Neville Russell,[3] Edward A. Atack,[3] Adele Quarrington,[3] and Leo Stolbach[2,3]

Primary central nervous system lymphoma is probably equivalent to lymphomas arising in other sites (1,2). The most common histopathologic type of brain lymphoma is the diffuse histiocytic variant (1). Improvement frequently follows cranial irradiation, but local relapse is frequent and long-term survival is very rare (2,3). There is little information regarding this neoplasm's responsiveness to chemotherapy. Corticosteroids (4), high-dose methotrexate iv with folinic acid rescue (5), and intrathecal methotrexate (6) have each produced objective regressions of primary central nervous system lymphomas.

One of the most active chemotherapeutic regimens against extracerebral lymphomas is a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (7). There are no reports of its use in primary brain lymphomas.

CASE REPORT

On December 28, 1981, a 63-year-old woman was admitted with a 10-day history of progressive weakness leading to inability to walk, personality change, and urinary incontinence. Physical examination revealed a severe encephalopathy with frontal features predominating. A computerized tomographic (CT) scan of the brain (fig 1) showed a massive midline contrast-enhancing lesion extending throughout the corpus callosum and almost filling the left lateral ventricle. No neurologic improvement was noted with dexamethasone. A craniotomy on January 8, 1982, revealed a diffuse histiocytic lymphoma. A left frontal lobectomy was performed, leaving behind grossly visible tumor.

Postoperatively, the patient remained unchanged neurologically. Staging procedures showed no evidence of extracerebral lymphoma. On January 16, 1982, the patient was given chemotherapy consisting of cyclophosphamide (750 mg/m^2 iv), doxorubicin (50 mg/m^2 iv), vincristine (2 mg iv), and dexamethasone (8 mg iv). Within 4 days, substantial neurologic improvement was noted. A CT scan performed on January 19, 1982, revealed a marked reduction in the contrast-enhanced mass, including posterior areas where no tumor had been removed surgically (fig 2). The left lateral ventricle remained obliterated, and extensive cerebral edema remained in the left hemisphere. A CT scan had not been done between the time of surgery and initiation of chemotherapy. On January 26, 1982, the patient became febrile while neutropenic but recovered with antibiotics. A second course of chemotherapy was given on February 4, 1982, with a 20% reduction in the dose of cyclophosphamide and doxorubicin. A CT scan done February 9, 1982, showed further marked improvement, with decreased mass effect, almost complete resolution of contrast enhancement, and reconstitution of the middle and posterior portions of the left lateral ventricle (fig 3); further neurologic improvement was noted. On February 23, 1982, cranial irradiation was initiated without further change in the patient's condition. Three additional courses of chemotherapy were given, and all therapy was discontinued in June 1982. At the time of last follow-up in October 1982 there was no evidence of lymphoma recurrence clinically or on CT scan.

DISCUSSION

This patient with a primary diffuse histiocytic lymphoma of the brain apparently experienced a marked response to the iv administration of cyclophosphamide, doxorubicin, and vincristine and iv and oral dexameth-

[1]Received June 28, 1982; revised Dec 13, 1982; accepted Jan 6, 1983.

[2]Ontario Cancer Treatment and Research Foundation Clinic, Ottawa, Canada.

[3]Department of Medicine (*D. J. Stewart, E. A. Atack,* and *L. Stolbach*), Department of Surgery, Division of Neurosurgery (*N. Russell*), and Department of Radiology (*A. Quarrington*), University of Ottawa, Ontario, Canada.

**Reprint requests to:* David J. Stewart, MD, Ontario Cancer Foundation Clinic, 501 Smyth Rd, Ottawa, Ontario K1H 8L6, Canada.

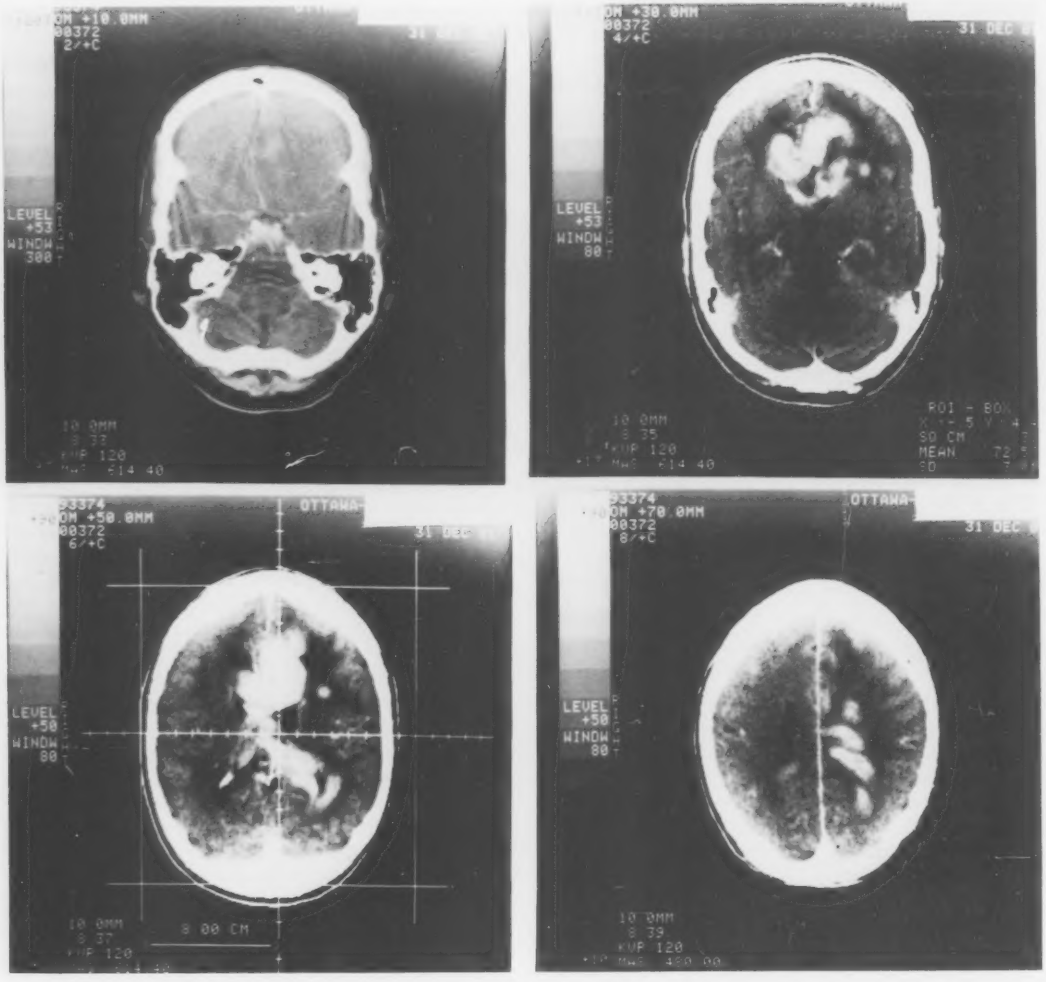


FIGURE 1.—CT brain scan prior to left frontal lobectomy in patient with primary brain lymphoma revealed massive midline contrast-enhancing lesion extending throughout corpus callosum and almost filling left lateral ventricle.

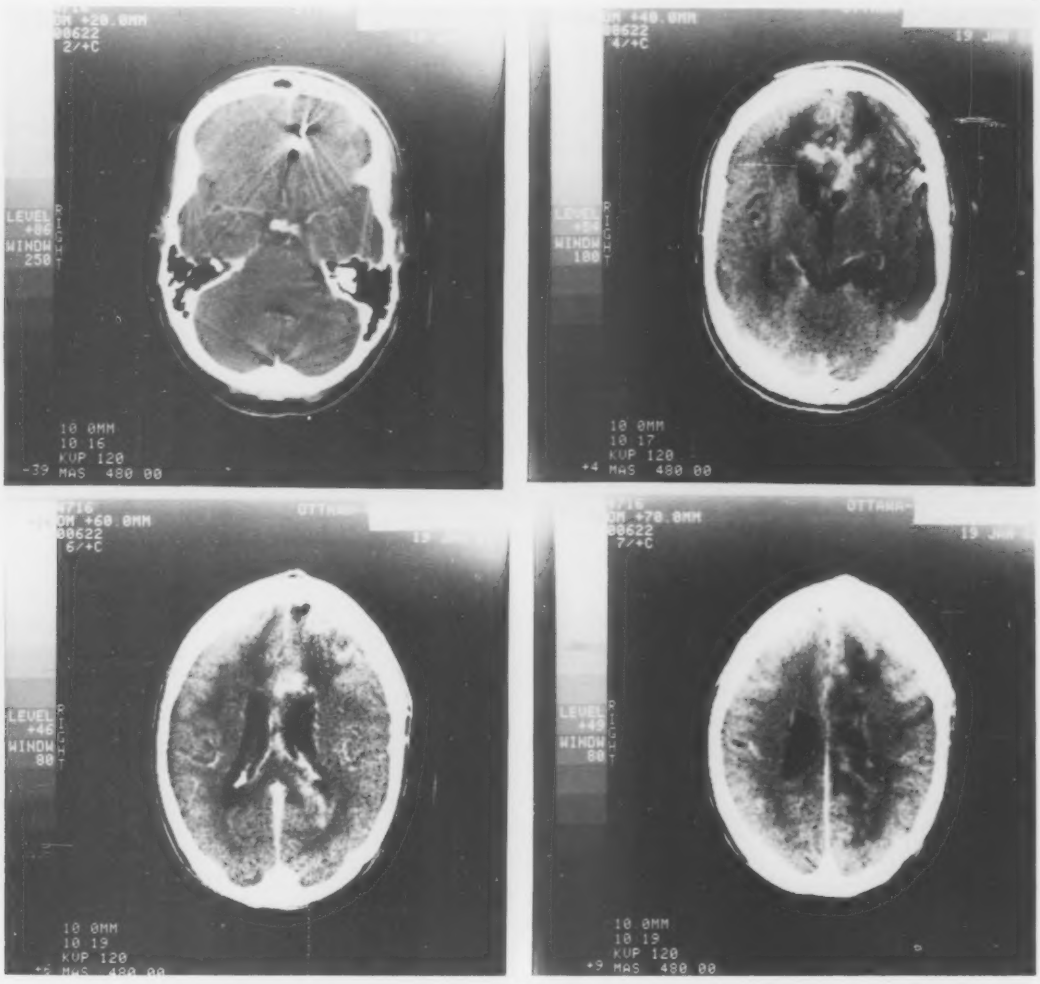


FIGURE 2.—CT scan was repeated 11 days after left frontal lobectomy and 3 days after chemotherapy with cyclophosphamide, doxorubicin, vincristine, and dexamethasone. No tumor had been resected from posterior area. Scan demonstrated marked reduction in contrast-enhanced mass, but left ventricle remained obliterated.

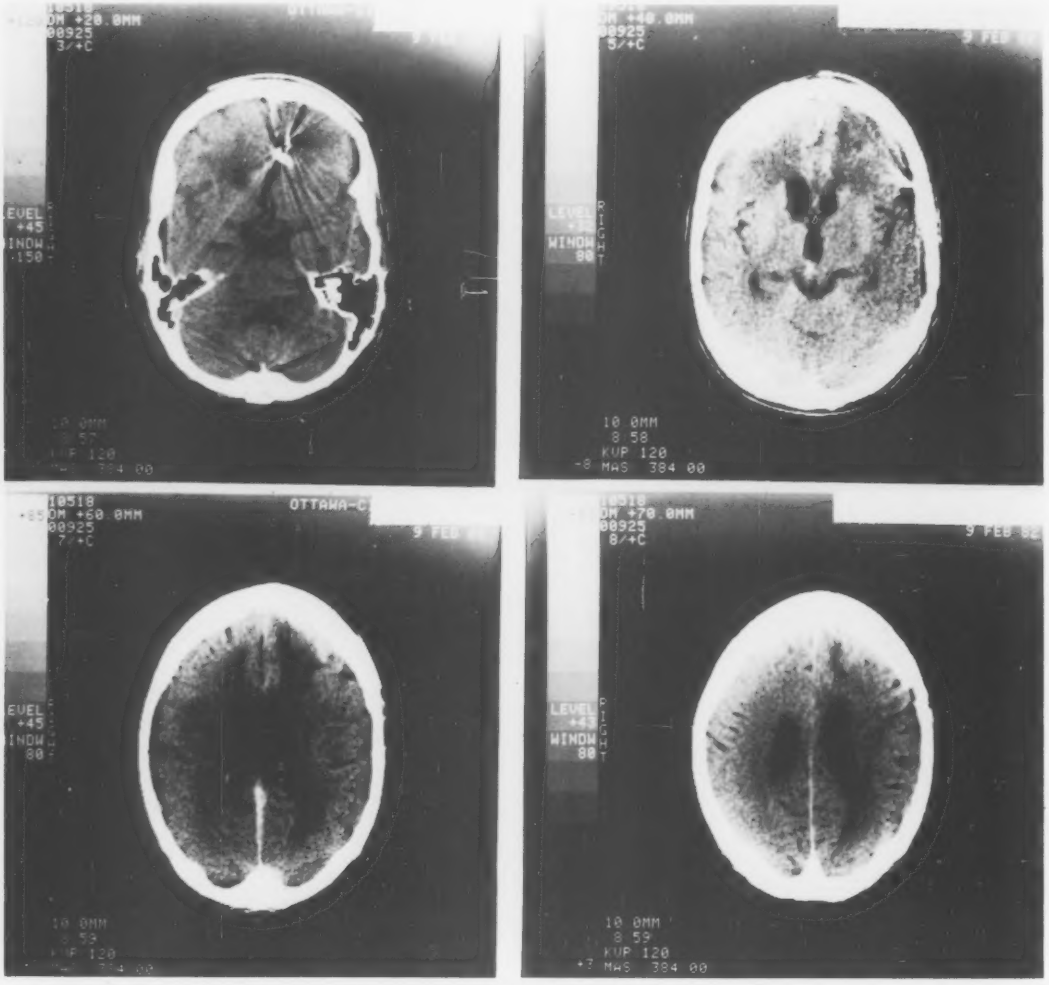


FIGURE 3.—CT scan 24 days after 1st course of chemotherapy and 5 days following 2nd course revealed further marked improvement, with decreased mass effect, almost complete resolution of contrast enhancement, and reconstitution of middle and posterior portions of left lateral ventricle.

asone. It is known that primary brain lymphomas may respond to corticosteroids (4), and it is possible that dexamethasone alone was responsible for the observed therapeutic effect. Despite this possibility, we feel it is very important that this chemotherapy combination be investigated more thoroughly in patients with primary brain lymphomas. Survival > 5 years is rare in patients with primary brain lymphomas (2,3), but using this chemotherapy combination, cure is possible for some types of extracerebral lymphomas (7).

The blood-brain barrier is disrupted to a variable degree within intracerebral tumors (8), and many chemotherapy agents that do not attain high concentrations in normal brain are capable of inducing objective regression of intracerebral tumors (9–11). Systemically administered chemotherapeutic agents generally attain high concentrations in human brain tumors (12–14). It is possible that chemotherapy-induced cure may be less likely in brain lymphomas than in extracerebral lymphomas, since small, isolated tumor deposits may occur far from the main body of the tumor (2), in areas where a more intact blood-brain barrier could limit drug penetration.

Nevertheless, we feel that the combination of cyclophosphamide, doxorubicin, vincristine, and corticosteroids should be further investigated in this disease in view of the apparent benefit derived by our patient.

REFERENCES

1. Taylor CR, Russell R, Lukes RJ, et al. An immunohistologic study of immunoglobulin content in primary central nervous system lymphomas. Cancer 41:2197–2205, 1978.
2. Jellinger K, Rodeskiewicz T, and Slowik F. Primary malignant lymphomas of the central nervous system in man. Acta Neuropathol (Berlin) Suppl VI:95–102, 1975.
3. Littman P, and Wang CC. Reticulum cell sarcoma of the brain: a review of the literature and a study of 19 cases. Cancer 35:1412–1420, 1975.
4. Williams RS, Crowell RM, Fisher M, et al. Clinical and radiological remission in reticulum cell sarcoma of brain. Arch Neurol 36:206–210, 1979.
5. Ervin T, and Canellos GP. Successful treatment of recurrent primary central nervous system lymphoma with high-dose methotrexate. Cancer 45:1556–1557, 1980.
6. Herbst KD, Corder MP, and Justice GR. Successful therapy with methotrexate of a multicentric mixed lymphoma of the central nervous system. Cancer 38:1476–1478, 1976.
7. McKelvey EM, and Moon TE. Curability of non-Hodgkin's lymphoma. Cancer Treat Rep 61:1185–1190, 1977.
8. Groothuis DR, Fischer JM, Vick NA, et al. Comparative permeability of different glioma models to horseradish peroxidase. Cancer Treat Rep (suppl 2)65:13–18, 1981.
9. Khan A, and McCullough D. Use of cisplatinum in CNS malignancy. Proc Am Assoc Cancer Res and ASCO 20:336, 1979.
10. Sklansky BD, Mann-Kaplan RS, Reynolds AF, et al. 4'-Demethyl-epipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.
11. Taylor SG, Nelson L, Baxter D, et al. Treatment of grade III and IV astrocytoma with dimethyl triazeno imidazole carboxamide (DTIC, NSC-45388) alone and in combination with CCNU (NSC-79037) or methyl CCNU (MECCNU, NSC-95451). Cancer 36:1269–1276, 1975.
12. Stewart DJ, Leavens M, Friedman J, et al. Penetration of *N*-(phosphonacetyl)-L-aspartate into human central nervous system and intracerebral tumor. Cancer Res 40:3163–3166, 1980.
13. Stewart DJ, Leavens M, Maor M, et al. *Cis*-diamminedichloroplatinum: human central nervous system distribution and use as a radiosensitizer in malignant brain tumors. Cancer Res 42:2474–2479, 1982.
14. Rosenblum M, Stewart DJ, Yap BS, et al. Penetration of methylglyoxal bis(guanylhydrazone) into intracerebral tumors in humans. Cancer Res 41:459–462, 1981.

Phase II Study of Vinblastine in Small Cell Carcinoma of the Lung[1,2]

Sverre Sörenson,[3,*] Kell Østerlind,[4] Per Dombernowsky,[5] and Heine H. Hansen[4]

Thirty-five patients with small cell carcinoma of the lung, refractory to previous combination chemotherapy including vincristine, were treated with weekly iv bolus injections of vinblastine. The dose was 4 mg/m² in the first 22 patients and 7 mg/m² in the last 13. No responses were observed among 34 evaluable patients. Side effects included moderate leukopenia and minimal neurotoxicity. It is concluded that vinblastine appears to be inferior to other vinca alkaloids in the treatment of small cell carcinoma of the lung. [Cancer Treat Rep 67:293–295, 1983]

Combination chemotherapy is currently the basic form of treatment for small cell carcinoma of the lung (SCCL). At least ten chemotherapeutic agents are demonstrably active in this tumor type, and the superiority of certain combined regimens is well-established (1).

Among the vinca alkaloids, vincristine has been widely used as a part of combined treatment programs. The activity of vincristine was documented in a phase II study (2), and its value in combination chemotherapy was subsequently proven in a prospective randomized trial (3). Recent data indicate that the related drug, vindesine, is also active in heavily pretreated patients, with a response rate of about 25% (4,5). For a third vinca alkaloid, vinblastine, only little and inconclusive information is available (6,7). For this reason we conducted a phase II study of this drug in SCCL.

PATIENTS AND METHODS

Criteria for entry in the study included a histologic or cytologic diagnosis of SCCL; no prior treatment with vinblastine; no anticancer chemotherapy or irradiation for the previous 2 weeks (6 weeks for nitrosourea); favorable World Health Organization performance status (0–2) (8); wbc count $\geq 3000/mm^3$ and platelet count $\geq 100{,}000/mm^3$, unless bone marrow metastases were documented; measurable or evaluable disease; and informed consent. All patients had previously received combination chemotherapy including vincristine, with or without radiotherapy.

Before treatment with vinblastine, all patients had a complete medical history, physical examination, chest roentgenogram, bone marrow examination, and laboratory evaluation including a cbc. Serum chemistries included creatinine, bilirubin, SGOT, LDH, and alkaline phosphatase. During treatment, peripheral blood cell counts were obtained once weekly, while physical examination, chest roentgenogram, serum creatinine, and liver chemistries were repeated every 2 weeks.

The disease was classified as limited if clinically detected tumor was confined to one lung, the mediastinum, and supraclavicular lymph nodes, while tumor involvement beyond these regions was classified as extensive disease.

Vinblastine was administered by rapid iv injection. The initial dose was 4 mg/m² once weekly in the first 22 patients. When it became apparent that the hematologic toxicity was modest, the dose was escalated to 7 mg/m² in the remaining 13 patients. The subsequent doses were modified according to peripheral blood cell counts. If moderate neurotoxicity (constipation, pain, paresthesias, and muscular weakness) occurred, the dose was reduced by 50%. The drug was completely withdrawn if symptoms were severe or if toxicity increased despite dose reduction.

Treatment was continued until objective evidence of

[1]Received July 6, 1982; revised Nov 19, 1982; accepted Dec 21, 1982.
[2]Supported by a grant from The Esper and Olga Boel Foundation.
[3]Department of Pulmonary Medicine, Renströmska Hospital, Göteborg, Sweden.
[4]Department of Chemotherapy RII–V, Finsen Institute, Copenhagen, Denmark.
[5]Medical Department C, Bispebjerg Hospital, Copenhagen, Denmark.
Reprint requests to: Sverre Sörenson, MD, Department of Pulmonary Medicine, Renströmska Hospital, Box 17301, S-402 64 Göteborg, Sweden.

disease progression or unacceptable toxicity occurred. Standard criteria for definition of tumor regression and progression were employed (8).

RESULTS

The characteristics of the patients, including data on previous therapy, are summarized in table 1. One patient refused further vinblastine treatment after the first injection and was not considered evaluable. Among the remaining 34 patients, no tumor response was observed. Data about treatment duration and side effects are presented in table 2. Moderate leukopenia was the most common form of toxicity. Neurotoxicity was observed in four patients. The symptoms were mild and consisted of paresthesias (one patient), muscular pain (one), and constipation (two).

DISCUSSION

The results obtained with vinblastine in this study were disappointing. No tumor regressions were observed despite a dosage that induced a moderate degree of hematologic toxicity.

Heavy previous chemotherapy in all patients may, in part, explain our failure to demonstrate any therapeutic activity of the drug. The possibility of cross-resistance

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	35
Age (yrs)	
Median: 60	
Range: 41–74	
Males/females	27/8
Performance status*	
0	0
1	18
2	17
Extent of disease	
Limited	22
Extensive	13
Pretreatment weight loss	25
Prior chemotherapy (3–7 drugs)	35
Vincristine†	35
Vindesine	1
Prior radiotherapy	6
Indicator lesion	
Lung	34
Mediastinum	14
Lymph nodes	6
Liver	2
Skin	3

*World Health Organization scale.
†Median dose, 13.5 mg (range, 4–43.5).

TABLE 2.—Results of treatment

	Initial dose level	
	4 mg/m^2	7 mg/m^2
No. of evaluable patients	22	12
Duration of treatment (days)		
Median	29	36
Range	12–57	21–64
No. of doses		
Median	4	4
Range	1–7	1–8
% of planned total dose given		
Median	83	60
Range	41–167	25–98
No. of responses	0	0
Leukocyte count nadir (cells × 10^3/mm^3)		
Median	2.7	2.1
Range	1.0–6.9	0.8–6.7
Platelet count nadir (cells × 10^3/mm^3)		
Median	186	163
Range	61–622	55–339
No. of febrile episodes during leukopenia	0	0
No. of patients with neurotoxicity	3	1

between vincristine and vinblastine should also be considered. In malignant lymphoma, however, these drugs do not appear to be cross-resistant (9). The experience with vindesine, which is obviously active in patients with SCCL who have had extensive prior chemotherapy including vincristine (4,5), suggests that differences in the antineoplastic spectrum of vinca alkaloids is a more likely explanation for the negative findings.

In patients who respond to weekly injections of vindesine, tumor regression is generally observed within 1 or 2 weeks from the start of therapy (5). For this reason, patients in the present study were evaluated for tumor response even if the duration of treatment was only 2 weeks (minimum duration, 12 days). More restrictive criteria for evaluation would clearly not have changed the conclusions.

Our patients were treated with weekly iv injections of the drug. Other modes of administration may, however, have a more favorable therapeutic index. Pharmacokinetic studies have shown that after iv bolus administration of vinblastine, the drug is rapidly cleared from the blood in a triphasic manner (10). Continuous 5-day infusions of vinblastine have induced responses in patients with breast cancer who are refractory to intermittent injections with the same drug (11). Similar schedule dependency has been reported for vincristine (12) and vindesine (13).

Although a comparative trial has not been carried out, the results from the present study strongly suggest that in the treatment of SCCL, vinblastine is inferior to the other vinca alkaloids, vincristine and vindesine. With some reservations for other modes of administration, it may be concluded that the incorporation of vinblastine in combined regimens containing vincristine is unlikely to improve therapeutic results to any considerable extent in this tumor type.

REFERENCES

1. Sørenson S. Treatment of small cell carcinoma of the lung. Eur J Respir Dis 62:315–331, 1981.
2. Dombernowsky P, Hansen HH, Sørensen PG, et al. Vincristine (NSC-67574) in the treatment of small-cell anaplastic carcinoma of the lung. Cancer Treat Rep 60:239–242, 1976.
3. Hansen HH, Dombernowsky P, Hansen M, et al. Chemotherapy of advanced small-cell anaplastic carcinoma. Superiority of a four-drug combination to a three-drug combination. Ann Intern Med 89:177–181, 1978.
4. Natale RB, Gralla RJ, and Wittes RE. Phase II trial of vindesine in patients with small cell lung carcinoma. Cancer Treat Rep 65:129–131, 1981.
5. Østerlind K, Dombernowsky P, Sørensen PG, et al. Vindesine in the treatment of small cell anaplastic bronchogenic carcinoma. Cancer Treat Rep 65:245–248, 1981.
6. Crosbie WA, Kamdar HH, and Belcher JR. A controlled trial of vinblastine sulphate in the treatment of cancer of the lung. Br J Dis Chest 60:28–35, 1966.
7. Wright TL, Hurley J, Korst DR, et al. Vinblastine in neoplastic disease. Cancer Res 23:169–179, 1963.
8. WHO Handbook for Reporting Results of Cancer Treatment. WHO publication No. 48, Geneva, 1979.
9. Carbone PP, Bono V, Frei E, III, et al. Clinical studies with vincristine. Blood 21:640–647, 1963.
10. Nelson RL, Dyke RW, and Root MA. Comparative pharmacokinetics of vindesine, vincristine, and vinblastine in patients with cancer. Cancer Treat Rev 7:17–24, 1980.
11. Yap H-Y, Blumenschein GR, Keating MJ, et al. Vinblastine given as a continuous 5-day infusion in the treatment of refractory advanced breast cancer. Cancer Treat Rep 64:279–283, 1980.
12. Jackson DV, Jr, Sethi VS, Spurr CL, et al. Intravenous vincristine infusion: phase I trial. Cancer 48:2559–2564, 1981.
13. Yap H-Y, Blumenschein GR, Bodey GP, et al. Vindesine in the treatment of refractory breast cancer: improvement in therapeutic index with continuous 5-day infusion. Cancer Treat Rep 65:775–779, 1981.

Use of High-Dose Oral Methotrexate Sequenced at 24 Hours With 5-FU: A Clinical Toxicity Study[1,2]

Chris Benz, Mike Silverberg, and Ed Cadman[3,*]

Twenty-three patients with advanced carcinoma were treated with 131 courses of high-dose oral methotrexate (MTX), sequenced at 24 hours with 5-FU iv and subsequent leucovorin rescue. The 30% incidence of toxicity was predominantly mild to moderate mucositis and myelosuppression. Trough and peak serum MTX levels demonstrated that micromolar concentrations were sustained > 24 hours. Toxicity correlated with shorter re-treatment intervals and not with serum MTX levels. This regimen can be safely and conveniently administered to an outpatient population and deserves further assessment in phase II trials. [Cancer Treat Rep 67:297–299, 1983]

Clinical trials of methotrexate (MTX) and 5-FU sequenced 1 hour apart in patients with breast and colorectal cancers have all shown encouraging responses with little if any toxicity (1–3). In vitro studies using human carcinoma cell lines have shown that optimal tumor toxicity is achieved by 18–24-hour sequencing of these drugs (4,5), but some in vivo studies have reported that MTX and 5-FU sequenced ≥ 4 hours apart produce unacceptable toxic effects in the host (6,7). In light of these reports, we designed a clinical toxicity study to assess whether MTX sequenced at 24 hours with 5-FU could be reasonably tolerated on an outpatient basis by patients with advanced malignancy.

PATIENTS AND METHODS

At the Yale-New Haven Hospital Chemotherapy Clinic, 23 patients with disseminated carcinoma received a total of 131 courses of MTX sequenced at 24 hours with 5-FU. Each patient had creatinine clearance ≥ 65 ml/minute, Hgb ≥ 11 g/dl, wbc count ≥ 4500/mm^3, and platelet count ≥ 200,000/mm^3. Prior chemotherapy had been given to 22 of the patients, including 17 who had previously received MTX sequenced at 1 hour with 5-FU, by a different protocol (1). The median interval from last chemotherapy was 4 weeks (range, 1–52). Toxicity and performance status were graded by Eastern Cooperative Oncology Group (ECOG) criteria (8) and are shown in table 1.

5-FU was obtained from Roche Laboratories (Nutley, NJ), and injectable MTX and leucovorin (LV) were obtained from the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Dose and schedule were: MTX, 50 mg/m^2 orally every 6 hours × 5; 5-FU, 600 mg/m^2 by iv bolus within 1 hour after the fifth dose of MTX; and LV, 10 mg/m^2 orally every 6 hours × 6, beginning 6 hours after the fifth MTX dose. The only dose modification was made for Patient 1, whose dose was reduced by 40% to reduce mucositis. Patients were initially re-treated at 7–14 days; if toxicity developed, the re-treatment interval was prolonged and drug dosage remained the same. Clinical and laboratory evaluations were performed prior to each treatment. Trough and peak serum MTX levels were measured by enzymatic inhibition of dihydrofolate reductase 1 hour before and 1 hour after the fifth dose of oral MTX (9).

RESULTS

Renal, liver, and central nervous system functions were not altered as a result of therapy. Overall toxicity occurred in 30% of 131 treatment courses, but specific

[1]Received July 9, 1982; revised Nov 18, 1982; accepted Dec 8, 1982.

[2]Supported by American Cancer Society grants CH-145 and CH-235, by Public Health Service grants CA-08341 and CA-27130 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, by a PMA Foundation Research starter grant, and by a Swebilius Cancer Research Award from Yale Comprehensive Cancer Center. Dr. Cadman is a recipient of a Faculty Cancer Research Award from the American Cancer Society.

[3]Departments of Medicine and Pharmacology, Yale University School of Medicine, New Haven, CT.

*_Reprint requests to:_ Ed Cadman, MD, Departments of Medicine and Pharmacology, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06510.

TABLE 1.—Patient characteristics and toxic effects

Characteristic			No. of treatment courses	No. of toxic courses (ECOG grade)						Extent of disease	ECOG status
							Myelosuppression				
Patient No.	Sex	Neoplasm		Nausea and vomiting	Diarrhea	Mucositis	Anemia	Leukopenia	Thrombocytopenia		
1	F	Breast	6	1 (2)	3 (1, 1, 1)	3 (2, 1, 1)	0	2 (1, 1)	0	Lung, bone, brain, abdomen	1
2	F	Breast	2	0	0	0	1 (1)	0	0	Skin, bone, lung	2
3	F	Breast	2	0	0	0	0	0	0	Bone	1
4	F	Breast	8	0	0	0	1 (1)	2 (2, 1)	0	Bone, brain	2
5	F	Breast	6	2 (2, 1)	2 (1, 1)	3 (2, 1, 2)	4 (2, 1, 1, 1)	3 (4, 1, 1)	0	Skin, nodes, lung, bone, liver	2
6	F	Breast	2	0	0	0	1 (1)	1 (3)	0	Skin, nodes, bone, meninges	3
7	F	Breast	13	0	1 (1)	2 (2, 1)	0	0	0	Skin, bone, lung, abdomen	1
8	F	Breast	5	0	0	0	0	0	0	Bone, meninges	1
9	F	Breast	6	0	0	0	0	0	0	Nodes	0
10	F	Breast	12	0	1 (1)	1 (1)	0	0	8 (1, 1, 2, 1, 1, 2, 1, 2)	Skin, lung, bone	1
11	F	Breast	9	0	0	0	0	0	0	Skin, nodes, lung	2
12	F	Colon	17	0	0	0	0	0	0	Abdomen, liver	1
13	M	Colon	5	1 (2)	0	0	1 (1)	0	1 (1)	Lung, bone, sinus	2
14	M	Colon	14	0	0	0	0	0	0	Liver	0
15	M	Colon	3	1 (1)	0	0	0	2 (1, 1)	0	Lung, liver	1
16	M	Colon	3	3 (1, 1, 1)	3 (2, 2, 2)	0	2 (1, 1)	2 (1, 1)	0	Liver, abdomen, bladder	1
17	M	Hepatoma	2	0	0	0	1 (2)	1 (2)	1 (2)	Liver, portal vein	1
18	F	Adenocarcinoma	5	0	2 (1, 1)	2 (1, 1)	0	0	0	Lung	0
19	F	Head and neck	3	0	0	1 (1)	2 (1, 1)	2 (1, 2)	0	Lung, esophagus	1
20	F	Head and neck	1	0	0	0	0	0	0	Skin, oral cavity	4
21	F	Mycosis fungoides	2	0	0	0	0	1 (1)	1 (1)	Skin	1
22	M	Mycosis fungoides	3	0	0	0	0	0	0	Skin	2
23	M	Mycosis fungoides	2	1 (2)	0	1 (1)	2 (1, 1)	2 (1, 1)	0	Skin, nodes	1
Specific toxicity*				7%	9%	10%	11%	14%	8%		

*Overall toxicity: 30% (38 of 131 courses).

TABLE 2.—Toxicity and serum MTX levels

	No. of treatment courses	Mean re-treatment interval (days)	Serum MTX (μM)*	
			Trough	Peak
Group A (15 patients)†				
Toxic effects	39	15	0.93 ± 0.54	1.99 ± 0.87
No toxic effects	35	21	0.88 ± 0.37	3.18 ± 2.68
Group B (8 patients)†				
No toxic effects	57	23	1.30 ± 0.85	2.65 ± 0.92
Total	131	20	1.20 ± 0.72	2.54 ± 1.2

*Values = mean ± SD.
†Group A includes patients who had toxic effects. Group B includes patients who had no toxic effects.

toxic effects occurred with an incidence < 15% (table 1). In the 15 patients experiencing toxicity, 31% of all toxic courses occurred with the first treatment; toxicity with repeated courses of therapy was controlled by prolonging the mean re-treatment interval from 15 to 21 days. The mean ECOG performance status was 1.4 for the entire patient group. No statistically significant difference was observed between the patient groups in table 2, although the mean age of Group B was slightly less than that of Group A (52 versus 58 years). Mean trough and peak MTX levels are listed according to toxicity in table 2. No significant correlation was observed between toxicity and serum MTX levels. Selected patients had serum MTX levels measured 1 hour after the initial oral dose. This mean value (1.1 ± 0.1 μM) fell within the peak and trough levels of the total group recorded in table 2, confirming that micromolar MTX concentrations were sustained over 24 hours, as previously reported (10). The large interpatient variation in MTX levels is also consistent with previous reports (10,11). Objective responses were observed in seven patients; however, because of multiple and varied treatments, including previous MTX sequenced at 1 hour with 5-FU in some patients, a more detailed analysis of responses would be inappropriate.

DISCUSSION

Administration of ≥ 250 mg/m² of MTX iv sustains micromolar serum levels for > 24 hours (12) and provides a concentration-time product that is more toxic than that of low-dose MTX (40 mg/m² iv) to colon and breast cancer cells treated in vitro (4,5). This clinical study confirms that high-dose oral MTX achieves and sustains micromolar blood levels (10), while also avoiding the extremely high peak concentrations observed with iv administration, which may contribute to MTX nephropathy (13).

When high-dose oral MTX is followed at 24 hours with 5-FU, toxic effects in the host are mild and generally acceptable. Leukopenia and mucositis each occurred with < 15% incidence; vomiting or diarrhea occurred with < 10% incidence. Toxicity did not correlate with MTX blood levels. Since high-dose oral MTX offers a safe, convenient, and alternative means of sequencing this drug with 5-FU in an outpatient population, further phase II studies appear to be warranted.

REFERENCES

1. GEWIRTZ A, and CADMAN E. Preliminary report on the efficacy of sequential methotrexate and 5-fluorouracil in advanced breast cancer. Cancer 47:2552–2555, 1981.
2. TISMAN G, and WU SJG. Effectiveness of intermediate-dose methotrexate and high-dose 5-fluorouracil as sequential combination chemotherapy in refractory breast cancer and as primary therapy in metastatic adenocarcinoma of the colon. Cancer Treat Rep 64:829–835, 1980.
3. CANTRELL JE, BRUNET R, LAGARDE C, ET AL. Phase II study of sequential methotrexate-5-FU therapy in advanced measurable colorectal cancer. Cancer Treat Rep 66:1563–1565, 1982.
4. BENZ C, and CADMAN E. Modulation of 5-fluorouracil metabolism and cytotoxicity by antimetabolite pretreatment in human colorectal adenocarcinoma, HCT-8. Cancer Res 41:994–999, 1981.
5. BENZ C, TILLIS T, TATTELMAN E, ET AL. Optimal scheduling of methotrexate and 5-fluorouracil in human breast cancer. Cancer Res 42:2081–2086, 1982.
6. SOLAN A, VOGL S, KAPLAN B, ET AL. 5-Fluorouracil and methotrexate in combination for colorectal cancer: unacceptable toxicity when intermediate dose MTX precedes FU by 4 hours. Proc Am Assoc Cancer Res and ASCO 21:335, 1980.
7. MULDER J, SMINK T, and VAN PUTTEN L. 5-Fluorouracil and methotrexate combination chemotherapy: the effect of drug scheduling. Eur J Cancer Clin Oncol 17:831–837, 1981.
8. OKEN M, CREECH R, TORNEY D, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Cancer Clin Trials, 1983. In press.
9. FINLEY PR, and WILLIAMS RJ. Methotrexate assay by enzymatic inhibition with use of the centrifuged analyzer. Clin Chem 23:2139–2141, 1977.
10. MCVIE JG, STUART JFB, CALMAN KC, ET AL. High-dose oral methotrexate. Cancer Treat Rep (suppl 1)65:141–143, 1981.
11. CRAFT AW, RANKIN A, and AHERNE W. Methotrexate absorption in children with acute lymphoblastic leukemia. Cancer Treat Rep (suppl 1)65:77–81, 1981.
12. HENDERSON E, ADAMSON R, and OLIVERIO V. The metabolic fate of tritiated methotrexate II. Absorption and excretion in man. Cancer Res 25:1018–1024, 1965.
13. BERTINO JR. Clinical use of methotrexate—with emphasis on use of high doses. Cancer Treat Rep (suppl 1)65:131–135, 1981.

Mitomycin-Associated Lung Disease[1]

Stanley R. Gunstream,[2,3] John J. Seidenfeld,[2,3,*] Richard E. Sobonya,[3] and Leo J. McMahon[2,3,†]

Over the past 20 years, mitomycin has been used in combination with other chemotherapeutic agents to treat various neoplasms. Mitomycin lung toxicity was first reported in the English literature in 1978, and presently there are approximately 13 reported cases (1–8). Since the introduction at our institution of a chemotherapeutic protocol for advanced lung cancer [incorporating mitomycin (10 mg/m^2 iv, Day 1), vincristine (2 mg iv, Day 1) or vindesine (3 mg/m^2 iv, Day 1), and 5-FU (300 mg/m^2, Days 1–4), given every 4–6 weeks], we have noted an increased incidence of mitomycin-associated lung disease.

METHODS

We carried out a retrospective review of patients who were referred to the Pulmonary Division, Veterans Administration Medical Center, from January 1980 to July 1981 with a history of having received mitomycin (10 mg/m^2). No patient who had received radiation therapy during the previous 2 years was included. Other causes of pulmonary disease were excluded during evaluation by the pulmonary consultant. From this group of approximately 200 patients, the records of six patients with clinical, radiographic, and pathologic features compatible with mitomycin-related lung disease are reviewed.

RESULTS

Patient characteristics are shown in table 1. Patient 5 received cyclophosphamide; however, this drug was discontinued 6 months prior to the onset of symptoms. Patient 6 received one course of cyclophosphamide and bleomycin after radiographic changes and dyspnea had developed.

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)/ sex	Mitomycin dose (mg)	No. of courses* before symptoms	Other chemotherapy†	Pathology‡
1	67/M	72	4	5-FU, DX (50 mg/m^2, 6 courses)	TBBX + A
2	39/F	59	4	5-FU, VDS	
3	56/M	76	4	5-FU, VCR	TBBX
4	78/M	40	3	5-FU, VCR	A
5	73/F	96	6	DX (60 mg/m^2, 4 courses) CY (15 mg/kg, 4 courses)	TBBX + A
6	55/M	80	4	5-FU, VDS CY (15 mg/kg)§ CP (20 mg/m^2)§ BL (15 mg/m^2)§	TBBX

*4–6 weeks between courses.
†DX = doxorubicin, VDS = vindesine, VCR = vincristine, CY = cyclophosphamide, CP = cisplatin, and BL = bleomycin.
‡TBBX = transbronchial biopsy; A = autopsy.
§Given on 1 occasion.

[1]Received July 26, 1982; revised Nov 24, 1982; accepted Dec 21, 1982.
[2]Department of Internal Medicine, Veterans Administration Medical Center, Tucson, AZ.
[3]University of Arizona College of Medicine, Arizona Health Sciences Center, Tucson.
†Deceased.
Reprint requests to: John J. Seidenfeld, MD, Pulmonary Disease Section (111A), Department of Internal Medicine, Veterans Administration Medical Center, Tucson, AZ 85723.

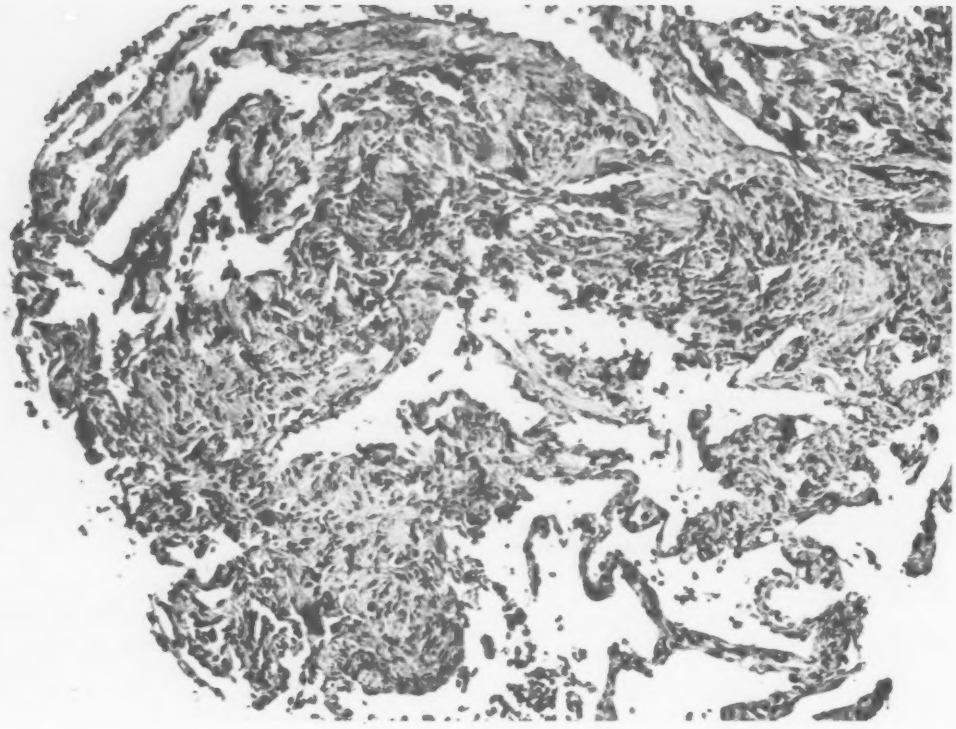


FIGURE 1.—Photomicrograph of the transbronchial biopsy in Patient 6, showing organizing inflammation and fibrosis; contrast the organizing inflammation in lower center with the adjacent relatively normal alveolar walls (hematoxylin and eosin stain, × 300).

Leukocyte counts were normal or low, and the differential counts showed no eosinophilia. Pulmonary function studies showed a restrictive pattern but most patients lacked baseline studies. All patients had some degree of hypoxemia at initial evaluation.

All patients were dyspneic either at rest or with exertion, and all had interstitial infiltrates on chest radiographs. Basilar crackles and symptomatic cough were noted in five patients, and pleural effusions were noted in two. Two patients had fever at initial evaluation but were afebrile within 2 days.

In three patients, prednisone (1 mg/kg) seemed to have a beneficial effect, resulting in either increased exercise tolerance or increased P_aO_2. Two patients with initial improvement deteriorated with dose reduction (down to 0.25 mg/kg).

Tissue specimens were obtained from five patients; two patients had both transbronchial biopsy and autopsy, two had only transbronchial biopsy, and one was autopsied without previous biopsy. The histologic appearance of lung in the transbronchial biopsy specimen (figure 1) and that in the autopsy specimen (figure 2) were similar. Alveolar septal fibrosis was present, associated with an organizing pneumonitis characterized by intra-alveolar masses of fibrous tissue, or Masson bodies. A variable cellular interstitial infiltrate was present, consisting of lymphocytes, histiocytes, and plasma cells, but this was not prominent. Macrophages were variably present in alveolar spaces, but were never prominent. In the transbronchial biopsies, type 2 alveolar cell hyperplasia was present without cytologic atypia. However, in the autopsy material mild to moderate cytologic atypia was noted. No cytologic atypia attributable to chemotherapy was seen in any of the other organs examined. Slides from the autopsy specimens showed no alterations of terminal nonrespiratory airways which could be attributed to the chemotherapy.

DISCUSSION

The acute reaction after a single dose of mitomycin, noted in two patients by Buzdar et al (3), was not seen in the present series, nor did we see patients without radiographic changes. All of our patients had a more indolent progression of pulmonary disease, and no patient became symptomatic with less than three courses.

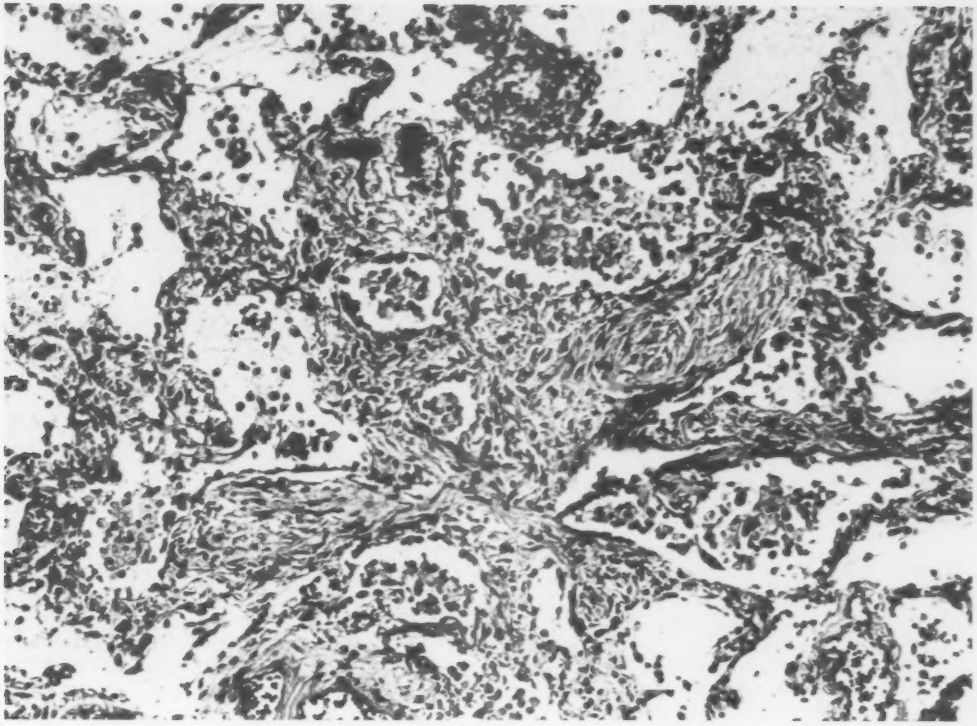


FIGURE 2.—Photomicrograph of interstitial and organizing pneumonia in Patient 1 at autopsy; masses of loose fibroblastic tissue representing alveolar organization are seen in the center (hematoxylin and eosin stain, × 300).

This may be due in part to the lower dose used (10 mg/m^2). Additionally, patients were referred because of pulmonary symptoms or radiographic changes and represent a select group, excluding those with a larger pulmonary reserve or mild impairment.

The lack of neoplasm in five of six of the pathologic specimens, as well as the clinical course subsequent to initial evaluation, was sufficient to rule out tumor recurrence as the etiology of progressive pulmonary disease. In the one case, autopsy showed nodules of bronchiolo-alveolar cell carcinoma in the lung but also showed diffuse interstitial and organizing pneumonitis. Likewise, all of our patients had complete evaluations for infection, including negative fungal and mycobacterial cultures. Viral cultures were not routinely done. Though viral illness associated with interstitial lung disease cannot be totally excluded, the pathologic material was not supportive of such etiology. No inclusion bodies were observed, nor was there focal inflammation or terminal airway abnormalities as often noted in viral pneumonitis.

Pleural disease has been previously reported but is not generally a part of most pulmonary drug reactions. Autopsy confirmed a lack of recurrent tumor in the chest in one of these patients; unfortunately, the other patient had no anatomic confirmation.

One feature not reported previously in mitomycin pneumonitis is intra-alveolar organization with fibrosis. Other histologic changes such as mild to moderate atypia of alveolar cells and sparing of terminal bronchioles are similar to changes described by others (1–3). It appears that marked cellular atypia as found in busulfan toxicity is not found with mitomycin. The fibrosis of alveolar septa with alveolar type 2 lining cell hyperplasia, as described by Yoon et al (4), was present in association with areas of organization.

CONCLUSION

We found evidence of mitomycin-associated lung disease in six patients over an 18-month period. Clinical, radiographic, and pathologic features were consistent with drug-induced injury. Our recent experience leads us to believe that mitomycin-associated lung disease may be more common than the 13 previously reported cases would suggest.

REFERENCES

1. Orwoll ES, Kiessling PJ, and Patterson JR. Interstitial pneumonia from mitomycin. Ann Intern Med 89:352–355, 1978.
2. Fielding JW, Crocker J, Stockley RA, et al. Interstitial lung disease in a patient treated with 5-fluorouracil and mitomycin C. Br Med J 2:551–552, 1979.
3. Buzdar AU, Legha SS, Luna MA, et al. Pulmonary toxicity of mitomycin. Cancer 45:236–244, 1980.
4. Yoon JM, Rao U, Vassilopoulos PP, et al. Interstitial pneumonia associated with mitomycin C and DTIC. Med Pediatr Oncol 9:61–67, 1981.
5. Andrews AT, Bowman HS, Patel SB, et al. Letter. Ann Intern Med 90:127, 1979.
6. Sostman HD, Matthay RA, and Putnam CE. Cytotoxic drug induced lung disease. Am J Med 62:608–615, 1977.
7. Krauss S, Sonoda T, and Solomon A. Treatment of advanced gastrointestinal cancer with 5-fluorouracil and mitomycin C. Cancer 43:1598–1603, 1979.
8. Weiss R, and Muggia F. Cytotoxic drug-induced pulmonary disease: update 1980. Am J Med 68:259–266, 1980.

PCNU in Advanced Breast Cancer[1,2]

Silvana Martino,* Bohumil A. Samal, and Amnuay Singhakowinta[3,4]

Breast cancer remains the most prevalent malignancy in American women. With combination chemotherapy, remission rates of 40%–82% can be achieved in advanced disease. However, remissions tend to be of short duration, and improvement in overall survival remains measurable in months. The need for new, effective agents is clear. PCNU, one of the *N*-(2-chloroethyl)-*N*-nitrosoureas, was evaluated in a phase II study.

PATIENTS AND METHODS

Twenty-two women with advanced stage IV breast cancer were treated with PCNU. All patients had received at least two prior chemotherapeutic trials, and 60% of them had received one or more hormonal treatments. Good-risk patients were treated with 100 mg/m^2 of PCNU given as a 30-minute iv infusion at 6-week intervals. Poor-risk patients received a dose of 75 mg/m^2. Only those patients who survived and were observed for a minimum of 6 weeks were considered evaluable for response.

RESULTS

Patient characteristics, response, and toxic effects are described in table 1. Seven patients, six of whom had a performance rating < 60%, had early deaths. Three died from progressive cancer, one from septicemia, and one from pulmonary emboli; two patients died suddenly within a few days of receiving PCNU, with no obvious cause.

Fifteen patients were evaluable for response. Four patients received two courses of PCNU, and 11 received one course followed by a 6-week observation period. No complete or partial responses were observed. All patients had progressive disease when they were removed from the study. Thrombocytopenia (13 of 15 patients), leukopenia (nine of 15), and nausea (seven of 15) were the major toxic effects. Additionally, one patient had mild dizziness, and one developed a mild skin rash on her back.

TABLE 1.—Characteristics of patients treated with PCNU

Characteristic	No. of patients
Total treated	22
Early death	7
Prior chemotherapy	22
Prior hormonal therapy	13
Evaluable	15
Response	0
Thrombocytopenia	13
Leukopenia	9

DISCUSSION

Previous experience with other nitrosoureas in breast cancer has demonstrated low but definite response rates (1). Because of its greater in vitro alkylating activity when compared to carmustine, lomustine (2), and semustine, we anticipated improved therapeutic results with PCNU.

Our experience in this group of heavily pretreated patients with advanced disease was disappointing but similar to that of other investigators. The Eastern Cooperative Oncology Group reported preliminary data on seven patients, none of whom had a response (3). Kavanagh et al (4), using slightly lower doses than our own (60–90 mg/m^2 at a 6-week interval) in 28 patients, observed one patient with mixed response and four with stable disease. It appears from the data available at present that PCNU does not have a role in the treatment of breast cancer.

REFERENCES

1. GOTTLIEB JA, RIVKIN SE, SPIGEL SC, ET AL. Superiority of adriamycin over oral nitrosoureas in patients with advanced breast carcinoma. Cancer 33:519–525, 1974.

[1]Received July 26, 1982; revised Nov 24, 1982; accepted Dec 21, 1982.
[2]Supported by contract N01-CM-07404 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Division of Oncology, Wayne State University, Detroit, MI.
[4]We gratefully acknowledge the assistance of Mrs. Mary Ann Terranova and Mrs. Lynn MacLeod in the preparation of this paper.
**Reprint requests to:* Silvana Martino, Division of Oncology, Wayne State University, Rm 520 Hudson, 3990 John R, Detroit, MI 48201.

2. Clinical Brochure. PCNU (NSC-95466). Bethesda, MD, National Cancer Institute, July 1978.
3. PCNU Minutes of the Biochemical Modulators Advisory Group Meeting. Phase I–II Working Group Meeting and New Drug Liaison (Phase II) Meeting. Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, Dec 15–16, 1981.
4. KAVANAGH JJ, YAP HY, BLUMENSCHEIN GR, ET AL. Phase II trial of PCNU in refractory advanced breast cancer. Am J Clin Oncol 5:249–251, 1982.

Phase II Trial of Bisantrene in Advanced Colorectal Carcinoma[1,2]

Tauseef Ahmed, Nancy E. Kemeny,* Richard A. Michaelson, and Harry D. Harper[3]

Bisantrene is a guanidino-substituted anthracene derivative with significant activity in several animal tumors. In vitro it inhibits thymidine uptake three to four times more actively than doxorubicin. The exact mechanism of action is not known, although related compounds intercalate into the DNA structure. Von Hoff et al (1) recommended administering bisantrene at a dose of 260 mg/m^2 iv every 3 weeks, after noting transient leukopenia, infusion-related hypotension, and phlebitis. It appears that bisantrene is not cardiotoxic, is less myelosuppressive than doxorubicin, and is not cross-resistant to doxorubicin.

MATERIALS AND METHODS

Twenty-five patients with measurable, histologically confirmed, advanced colorectal carcinoma were entered in this study. Patient characteristics are outlined in table 1.

The drug was mixed in 5% dextrose in water and infused over 90 minutes while blood pressure was monitored. The starting dose was 260 mg/m^2 iv every 3 weeks; if tolerated, this dose was escalated by 20 mg/m^2.

Response definitions included: *complete response*—the disappearance of all evidence of tumor; *partial response*—a > 50% decrease in the summed products of the perpendicular diameters of bidimensionally measurable lesions; *minor response*—a 25%–49% decrease; and *stable disease*—a < 25% change for 1 month. An adequate drug trial comprised two doses of the drug, with toxicity.

TABLE 1.—Patient characteristics

	No. of patients (%)
Total	25
Sex	
Males	15
Females	10
Age in yrs	
Median: 59	
Range: 38–73	
Performance status	
Median: 80	
Range: 60–100	
No prior chemotherapy	9
No prior irradiation	20
Indicator lesions	
Liver	17 (68)
Lung	6 (24)
Abdominal mass	5 (20)
Lymph nodes	2 (8)
Toxic effects	
Fever	21 (84)
> 104°F	5 (20)
Fatigue	9 (36)
Nausea/vomiting	8 (32)
Phlebitis	7 (28)
Sweating	4 (16)
Shaking chills	3 (12)
Midsternal pain	1 (4)
Transient hypotension	1 (4)
Multifocal PVCs*	1 (4)
Hyperglycemia	1 (4)
Olfactory hallucinations	1 (4)

*PVC = premature ventricular contraction.

RESULTS

Twenty-five patients received 42 infusions at a dose of 260 mg/m^2; this was increased to 280 mg/m^2 in ten patients and to 300 mg/m^2 in one. Further escalations were not attempted because most patients failed after two or three treatments.

Twenty-three patients had an adequate trial; two had an inadequate trial because of toxic effects. Three patients had stable disease after two to four doses but were unable to continue receiving the drug because of fever; 20 had progressive disease. Three of 18 patients receiving subsequent chemotherapy had a response.

[1]Received July 30, 1982; revised Dec 3, 1982; accepted Dec 21, 1982.
[2]Supported by contract N01-CM-97274 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.
Reprint requests to: Nancy E. Kemeny, MD, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

All patients were evaluable for toxic effects; the most common toxic effect was fever, which often started with the first dose. The median nadir wbc count was 3200/mm^3 (range, 1500–8200). Neither escalating the dose nor abnormal liver function tests affected the nadir wbc count. Chemical phlebitis was observed in seven patients. Drug toxicity was not cumulative. Significant alopecia, congestive heart failure, and mucositis were not seen.

Other investigators (2) have published data supporting our observations. With a predicted response rate of 15% ($P = 0.05$) we conclude that bisantrene at this dose and schedule is not an effective single agent in the treatment of colorectal carcinoma.

REFERENCES

1. Von Hoff DD, Myers W, Kuhn J, et al. Phase I clinical investigation of 9,10-anthracene dicarboxyaldehyde bis((4,5-dihydro-1*H*-imidazol-2-yl) hydrazone) dihydrochloride (CL 216,942). Cancer Res 41:3118–3121, 1981.
2. Perry MC, Forastiere AA, Richards F, et al. Phase II trial of anthracenedicarboxyaldehyde (ADC) in advanced colorectal cancer; a CALGB study. Proc ASCO 1:102, 1982.

Phase II Study of AMSA in Refractory Testicular Cancer[1,2]

Stephen D. Williams,[3,*] Paul Duncan,[4] and Lawrence H. Einhorn[3]

The recent successes in the management of germ cell tumors are well-known (1). However, some patients become refractory to treatment; their survival is measured in weeks.

AMSA is an acridine derivative which has shown activity in acute leukemia (2). It, however, has been devoid of activity in solid tumors (3). One possible explanation for the disparity between the response rates in leukemia versus solid tumors is that leukemia dosages have been substantially higher. Accordingly, this agent was chosen for evaluation in patients with refractory germ cell tumors. Patients having disease progression within 8 weeks of cisplatin therapy would receive AMSA alone; other patients not refractory to cisplatin would receive cisplatin plus AMSA. In all, AMSA dosages would approximate those found effective in acute leukemia.

PATIENTS AND METHODS

Six of nine patients were treated with AMSA alone and three with AMSA plus cisplatin. All patients were considered incurable and gave their informed consent. The patient population and treatment regimens are described in table 1. Courses were given every 3 weeks.

RESULTS

All six patients receiving AMSA alone received only one course and had disease progression. The three patients receiving cisplatin plus AMSA received two to three courses and had disease progression within 9 weeks. Median survival was 2 months.

As expected, hematologic toxicity was severe; it is described in table 1. There were two febrile episodes and one patient had bacteremia. Cardiac toxicity was not seen and there were no drug-related deaths.

DISCUSSION

In this study, therapeutic efficacy was not seen despite dosages adequate to cause severe toxicity. These results are similar to those of the European Organization on Treatment of Cancer (EORTC), which found no responses among 13 patients with testis tumor employing conventional-dose AMSA (90–120 mg/m^2 q 3

TABLE 1.—Patients and results

	No. of patients
Total*	9
Median age in yrs (range): 28 (19–46)	
Prior therapy†	
Cisplatin, vinblastine, bleomycin ± doxorubicin	8
Cisplatin + etoposide	6
Cyclophosphamide, vinblastine, bleomycin, cisplatin, and dactinomycin (VAB-6)	1
Other	3
Disease sites	
Lung	7
Liver	2
Nodes	3
Bone	1
Treatment	
AMSA (100 mg/m^2 daily × 3–4 days)	6
Cisplatin (20 mg/m^2 daily × 5 days) + AMSA (100 mg/m^2 × 3 days)	3
Response	0
Toxicity	
Wbc count nadir < 1000 cells/mm^3	7
Platelet count nadir < 25,000 cells/mm^3	4

*All patients were at least ambulatory and capable of self-care.

†No. of patients receiving each regimen; most had received > 1 prior regimen.

[1]Received Sept 13, 1982; revised Dec 13, 1982; accepted Jan 6, 1983.

[2]Supported in part by contract M01-RR-00750 from the Division of Research Resources, National Institutes of Health, Department of Health and Human Services. Dr. Einhorn is an American Cancer Society clinical professor of oncology.

[3]Department of Medicine, Indiana University Medical Center, Indianapolis.

[4]Hematology Oncology Associates, Albuquerque, NM.

*Reprint requests to: Stephen D. Williams, MD, Indiana University Medical Center, Indiana University Hospital, 926 W Michigan, Rm N-582, Indianapolis, IN 46223.

weeks) (3). No responses among nine patients does not preclude activity but suggests that the response rate would be < 30%. No responses among 22 patients (ours plusthe EORTC study) suggest that the response rate is< 13%. We believe further studies of AMSA in such patients are not warranted.

REFERENCES

1. Einhorn LH, Williams SD, Troner M, et al. The role of maintenance therapy in disseminated testicular cancer. N Engl J Med 305:727–731, 1981.
2. Legha SS, Keating MJ, Zander AR, et al. 4′-(9-Acridinylamino) methanesulfon-*m*-aniside (AMSA): a new drug effective in the treatment of adult acute leukemia. Ann Intern Med 93:17–21, 1980.
3. de Jager R, Siegenthaler P, Dombernowsky P, et al. Phase II study of 4′-(9-acridinylamino)-methan sulfon-*m*-aniside (*m*-AMSA). Proc Am Assoc Cancer Res and ASCO 22:367, 1981.

Phase II Trial of ICRF-187 in Non-Small Cell Lung Cancer[1,2]

Ronald B. Natale[3,4,*] Richard H. Wheeler,[3] Marcia K. Liepman,[3,4] Anthony Sauder,[5] and Les Bricker[3]

ICRF-187 is the more soluble (+) enantiomer of the racemic mixture ICRF-159. It was developed for iv administration to overcome the questionable bioavailability of the orally administered ICRF-159 (1,2). These compounds are bis-diketopiperazine derivatives and possess unusual cytokinetic actions (3,4) and possible antimetastatic effects (5–7), protective action against anthracycline cardiotoxicity (8), and synergism with cisplatin (9), anthracyclines (10), and radiation therapy (11). ICRF-159 demonstrated clinical antitumor activity in patients with non-Hodgkin's lymphoma (12), non-small cell lung cancer (NSCLC) (13), breast cancer (14), and head and neck cancer (15). A phase I trial of ICRF-187 indicated that a dose of 1250 mg/m^2 daily for 3 consecutive days was well-tolerated, with dose-limiting toxicity consisting predominantly of reversible leukopenia and thrombocytopenia (16). We therefore conducted a disease-oriented phase II trial of ICRF-187 using this schedule in patients with advanced (stage $IIIM_1$) NSCLC.

MATERIALS AND METHODS

Twenty-nine patients with objectively measurable and histologically confirmed NSCLC were entered in the study. Performance status $\geq$ 50 (Karnofsky scale), initial wbc count $\geq$ 4000/mm^3 and platelet count $\geq$ 125,000/mm^3, normal renal function, serum bilirubin $<$ 3.0 mg/dl, no significant cardiac dysfunction, and informed consent were required for patient eligibility. At the start of therapy all patients had a complete history, physical examination, and laboratory evaluation including cbc and platelet count, 12-channel biochemical screening profile, serum electrolytes, BUN, serum creatinine, and chest roentgenogram. Radionuclide or computerized tomographic liver scans were obtained in patients with hepatomegaly and abnormal biochemical liver function tests, and radionuclide bone scans were obtained in patients with suspected bone metastases.

Each patient had a physical examination and a cbc and platelet count every 1–2 weeks. A biochemical screening profile, BUN and creatinine, chest roentgenogram, and any additional study required for tumor measurement were obtained every 3 weeks.

ICRF-187, supplied by the National Cancer Institute (Bethesda, MD), was administered by rapid iv infusion over 30–60 minutes. The initial dose was 1500 mg/m^2/day for 3 consecutive days. Treatment was continued on a 3-week basis in the absence of disease progression. Doses were increased by 300 mg/m^2 if the wbc count nadir was $>$ 3000/mm^3 and/or the platelet count nadir was $>$ 125,000/mm^3. Doses were decreased by 300 mg/m^2 if the wbc count nadir was $<$ 2000/mm^3 or the platelet count nadir was $<$ 75,000/mm^3.

An adequate trial required a minimum of one complete course of therapy and at least a 4-week survival. Therapeutic response criteria were defined as: *partial remission (PR)*—a $\geq$ 50% decrease of all measurable lesions $>$ 1 month; *minor response*—an objective tumor decrease of $>$ 25% but less than that required for a PR; and *disease progression*—a $<$ 25% change or objective increase in measurable disease or the appearance of new tumor lesions.

PATIENTS

Twenty-nine patients (23 males and six females) with a median age of 57 years (range, 36–78) and median Karnofsky performance status of 70 (range, 50–90) received ICRF-187. All patients had metastatic, objec-

[1]Received Oct 20, 1982; accepted Jan 6, 1983.

[2]Supported in part by contracts N01-CM-07405 (Division of Cancer Treatment, National Cancer Institute) and M01-RR-00042 (Division of Research Resources) from the National Institutes of Health, Department of Health and Human Services.

[3]Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan, Ann Arbor.

[4]Department of Internal Medicine, Division of Hematology/Oncology, Ann Arbor Veterans Administration Medical Center, MI.

[5]Department of Internal Medicine, Division of Pulmonary Diseases, University of Michigan, Ann Arbor.

[*]*Reprint requests to:* Dr. Ronald B. Natale, University of Michigan, Simpson Memorial Research Institute, 102 Observatory, Ann Arbor, MI 48109.

TABLE 1.—Myelotoxicity/course

	No. of courses with toxicity at a dose of—		
	1200 mg/m^2	1500 mg/m^2	1800 mg/m^2
Wbc count (cells/mm^3)			
> 4000	3	20	5
3000–3999	2	8	1
2000–2999	4	3	—
1000–1999	—	6	—
< 1000	—	1	—
Platelet count (cells/mm^3)			
> 125,000	6	30	6
100,000–124,999	1	3	—
75,000–99,999	1	—	—
50,000–74,999	1	3	—
< 50,000	—	2	—

tively measurable NSCLC including adenocarcinoma in 16 patients, squamous cell carcinoma in 11, and large cell undifferentiated carcinoma in two. Eighteen patients had not received prior chemotherapy; 11 patients had received prior radiation therapy, including cranial irradiation in seven for brain metastases, and five had undergone prior surgery. Documented sites of tumor involvement included lung (29 patients), cervical or axillary nodes (11), bone (eight), brain (seven), and liver (three).

RESULTS

No objective responses occurred among 25 evaluable patients (predicted true response rate < 12%, $P = 0.05$), including 15 who had not received prior chemotherapy (predicted true response rate in chemotherapy-naive patients < 20%, $P = 0.05$). Three early deaths (two unrelated to drug or tumor) and one patient lost to follow-up account for the four inevaluable cases.

Fifty-five 3-day courses of ICRF-187 were administered to 26 patients who are evaluable for toxicity. Patients received a median of two courses (range, one to six). After the starting dose of 1500 mg/m^2, doses could be increased to 1800 mg/m^2 in four patients and to 2100 mg/m^2 in two; dose reductions to 1200 mg/m^2 were required in four patients and to 900 mg/m^2 in one. ICRF-187 was generally well-tolerated at these doses on this schedule, with dose-limiting toxicity consisting of reversible leukopenia and/or thrombocytopenia (table 1). The median wbc count nadir after the starting dose in 11 patients who had received prior chemotherapy compared to 15 patients who had not received prior chemotherapy was 3200 versus 5400/mm^3, respectively; the median platelet count nadir was 110,000 versus 306,000/mm^3, respectively. Other toxic effects included moderate alopecia (nine patients) and mild nausea and vomiting (eight). One patient developed transient mild elevations in SGOT, probably related to ICRF-187 as has been previously reported to occur in a few patients (16).

DISCUSSION

ICRF-159, administered orally for 3 consecutive days, was reported to have some, albeit low, activity in NSCLC (13). Since iv administered ICRF-187 provided a means to overcome the questionable bioavailability of ICRF-159, we tested its activity in patients with advanced, measurable NSCLC. Unfortunately, ICRF-187, administered on a daily × 3 schedule every 3 weeks, demonstrated no therapeutic activity in 25 patients with good performance status, 15 of whom had not received prior therapy. Due to the good performance status and lack of prior therapy in most patients, a starting dose 20% higher than what was recommended in the phase I study (16) was well-tolerated. We conclude that ICRF-187, in the dose and schedule used, is not active as a single agent in advanced bronchogenic carcinoma.

REFERENCES

1. CREAVEN PJ, ALLEN LM, and ALFORD DA. The bioavailability in man of ICRF-159, a new oral antineoplastic agent. J Pharm Pharmacol 27:914–918, 1975.
2. REITA AJ, BALTEZOR MJ, and BANSAL PC. Utilization of an enantiomer as a solution to a pharmaceutical problem: application to solubilization of 1,2-di(4-piperazine-2,6-dione) propane. J Pharm Sci 65:238–242, 1976.
3. SHARPE HBS, FIELD FO, and HELLMANN K. Mode of action of the cytostatic agent "ICRF-159." Nature 226:524–526, 1970.
4. WHEELER RH, NATALE RB, CLAUW D, ET AL. The cytotoxic and cytokinetic effects of ICRF-187 and ICRF-159 in vitro, and ICRF-187 in human bone marrow in vivo. Proc ASCO 1:13, 1982.
5. ATHERTON A. The effect of (±) 1,2-bis(3,5-dioxopiperazin-1-yl) propane (ICRF-159) on liver metastases from a hamster lymphoma. Eur J Cancer 11:383–388, 1975.

6. Hellmann K, and Burrage K. Control of malignant metastases by ICRF-159. Nature 224:273–275, 1969.
7. James SE, and Salsburg AJ. Effect of (±)-1,2-bis(3,5-dioxopiperazin-1-yl) propane on tumor blood vessels and its relationship to the anti-metastatic effect in the Lewis lung carcinoma. Cancer Res 34:839–842, 1974.
8. Herman EH, and Ferrous VJ. Reduction of chronic doxorubicin cardiotoxicity in dogs by pretreatment with (±)-1,2-bis(3,5-dioxopiperazinyl-1-yl) propane (ICRF-187). Cancer Res 41:3436–3440, 1981.
9. Woodman RJ. Enhancement of antitumor effectiveness of ICRF-159 (NSC-129943) against early L1210 leukemia by combination with *cis*-diamminedichloroplatinum (NSC-19875) or daunomycin (NSC-821151). Cancer Chemother Rep Part 2, vol 4(1):45–52, 1974.
10. Woodman RJ, Cysyk RL, Kline I, et al. Enhancement of the effectiveness of daunorubicin (NSC-82151) or adriamycin (NSC-123127) against early mouse L1210 leukemia with ICRF-159 (NSC-129943). Cancer Chemother Rep 59:689–695, 1975.
11. Hellmann K, and Murkin GF. Synergism of ICRF-159 and radiotherapy in treatment of experimental tumors. Cancer 34:1033–1039, 1974.
12. Flannery EP, Corder MP, Sheehan TF, et al. Phase II study of ICRF-187 in non-Hodgkin's lymphomas. Cancer Treat Rep 62:465–468, 1978.
13. Eagan RT, Carr DT, Cales DT, et al. ICRF-159 versus polychemotherapy in non-small cell lung cancer. Cancer Treat Rep 60:947–948, 1976.
14. Creech RH, Engstrom PF, Harris DT, et al. Phase II study of ICRF-159 in refractory metastatic breast cancer. Cancer Treat Rep 63:111–114, 1979.
15. Shah MK, Engstrom PF, Catalano RB, et al. Phase II trial of razoxane (ICRF-159) in patients with squamous cell carcinoma of the head and neck previously exposed to systemic chemotherapy. Cancer Treat Rep 66:557–558, 1982.
16. Von Hoff DD, Howser D, Lewis BJ, et al. Phase I study of ICRF-187 using a daily for 3 days schedule. Cancer Treat Rep 65:249–252, 1981.

Responder Versus Nonresponder Comparisons: Daunorubicin Plus Prednisone in Treatment of Acute Nonlymphocytic Leukemia[1]

In the report by Peterson et al in this journal (1), you have once again allowed misleading claims of efficacy to be based on the finding of longer survival times for responders to treatment as compared with nonresponders. Another example from your journal has been described by me elsewhere (2).

Peterson et al (1) conclude that their experience verifies the efficacy of daunorubicin plus prednisone in the treatment of acute nonlymphocytic leukemia, noting in their summary particular effectiveness relative to acute promyelocytic leukemia. An added claim is the demonstration that patients achieving complete remission have improved survival.

Specifically, during the years 1967–1977, 21 of 72 (29%) adults receiving daunorubicin plus prednisone achieved complete remission (six of nine [67%] patients with acute promyelocytic leukemia achieved complete remission). These 21 patients had a median survival time of 20 months compared with 2 months for the 51 nonresponders, with a median remission duration of 7 months (the six responders with acute promyelocytic leukemia showed a median remission duration of 29 months, with two of the six still in initial remission after > 4 years). Other factors such as patient age and hematologic features seemed prognostic of the occurrence of remission.

Without an actual control group, however, it cannot be said whether the study shows any therapeutic benefit for the treatment modality. If that treatment were completely ineffective, spontaneous responders would still be expected to outlive nonresponders. Even if treatment were, on balance, harmful, we might still expect responders to outlive nonresponders. Outcome for the entire group of 72 patients is the only positive evidence available.

These patients had an overall remission rate of 29%, an overall median survival time of 4 months, and an overall 4-year survival rate of 3%. This 3% represents two of the 72 patients studied, who were also two of the 21 responders and two of the six responders with acute promyelocytic leukemia. Peterson et al (1) note that other drugs given alone have yielded complete remission rates < 25% compared with < 50% for combination therapies, 20%–50% for daunorubicin alone, and 63%–85% for intensive chemotherapeutic regimens incorporating daunorubicin. Those more optimistic results were achieved in more recent studies.

Of the 51 nonresponders, 23 had died within 30 days of the initiation of therapy, with 21 of the 23 deaths due to sepsis and/or hemorrhage. It is quite possible that some of these 23 patients would have gone into remission had they lived longer. Therefore, a therapeutic regimen that reduced early deaths would elicit higher rates of remission, and therein could lie the success of the more recent intensive therapies.

That the occurrence of remission is prognostic of better survival is a reasonable interpretation. The longer duration of survival of responding patients may merely reflect that patients who had early death were automatically classified as nonresponders. To an extent, long survival increases the chances of remission rather than remission resulting in longer survival. Following initiation of therapy, the important question then is which patients will live longer, those already in remission or those not yet in remission (though they may enter remission at some later time)? The statistical methodology described by Mantel and Byar (3) would be appropriate for analyzing the data of Peterson et al (1). That remission is a favorable prognostic factor would then become a matter of demonstrated fact rather than a matter of faith. The Mantel and Byar approach could be applied so as to take into account age, or other factors as desired, within the limitations of the amount of data available. (An unavoidable complication would be that patients entering complete remission would receive maintenance therapy, so that we would not be able to distinguish between a remission effect and a maintenance therapy effect.)

The report by Peterson et al (1) leaves in doubt that the therapy reported on was efficacious, and it fails to bring out positive evidence that complete remission is prognostic of improved survival. By similar methods of analysis, Krebiozen and other dubious agents could undoubtedly be shown to be wonder drugs.

[1]Supported by Public Health Service grant CA-34096 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

REFERENCES

1. Peterson BA, Bloomfield CD, Theologides A, et al. Daunorubicin-prednisone in the treatment of acute nonlymphocytic leukemia. Cancer Treat Rep (Suppl 4)65:29–33, 1981.

2. MANTEL N. An uncontrolled clinical trial—treatment response or spontaneous improvement? Controlled Clin Trials 3:369–370, 1982.
3. MANTEL N, and BYAR DP. Evaluation of response-time data involving transient states: an illustration using heart-transplant data. J Am Stat Assoc 69:81–86, 1974.

Nathan Mantel

Department of Mathematics, Statistics, and Computer Science
The American University
4900 Auburn Ave
Bethesda, MD 20814

October 19, 1982

Phase II Study of Vindesine in Patients With Hodgkin's Disease, Lymphoma, and Breast Cancer

Vindesine, a vinblastine derivative, showed antitumor activity in animal tumors similar to that of vincristine but was less neurotoxic (1). In a phase II study at the National Cancer Institute, Cairo, we used vindesine to treat patients with Hodgkin's disease, lymphoma, and breast cancer, who were resistant to conventional lines of treatment or who had relapsed after initial response to therapy. There were 74 patients: 26 with stage III and IV Hodgkin's disease, 24 with stage III and IV lymphoma, and 24 with breast cancer. All patients had been treated previously with combination chemotherapy: MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) in Hodgkin's disease; COP (cyclophosphamide, vincristine, and prednisone) or CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) in lymphoma; and CMF (cyclophosphamide, methotrexate, and 5-FU) in breast cancer.

Vindesine was supplied in 5-mg vials by Eli Lilly and Co, Indianapolis. It was given at a dose of 3 mg/m^2/week by iv push through a running iv infusion. Patients were required to have adequate bone marrow, a leukocyte count $\geq$ 4000, and a platelet count $\geq$ 100,000. Dose was escalated to 3.5 mg/m^2/week if there was no response to therapy after four doses of vindesine. Patients were evaluated by clinical, laboratory, and radiologic examinations (if needed) every 2 weeks. Toxic effects were recorded weekly. The average number of cycles given was six, and the average total dose of drug per patient was 30 mg.

The results are summarized in table 1. The criteria of response were as follows: *CR*—the disappearance of all measurable lesions, with no new lesions, for at least 4 weeks; *PR*—a > 50% reduction in the size of measurable lesions, with no new lesions; *stable disease*—no change in measurable lesions, or the reduction of lesions by < 50%, with no new lesions; and *progressive disease*—a $\geq$ 25% increase in measurable lesions and/or the appearance of new lesions. Responses (CR + PR) were achieved in 50% of the patients with Hodgkin's disease, 25% of those with lymphoma (all with diffuse, poorly differentiated lymphocytic lymphoma), and 16.6% of those with breast cancer. One patient with Hodgkin's disease achieved a CR, which is still maintained (20+ months). Two patients with lymphoma achieved CR and then relapsed after 8 and 10 months, respectively. The median duration of PR was 6 weeks in patients with Hodgkin's disease, 4 weeks in those with lymphoma, and 7 weeks in those with breast cancer.

The toxic effects were gastrointestinal (grade 1 nausea and vomiting) in 35 patients, and alopecia (grade 1) occurred in 31. Neurotoxicity, which occurred in 46 patients, was less marked than that encountered with vincristine in the same patients. Leukopenia (grade 1) was detected in 12 patients. Drug administration was never stopped because of toxicity; all toxic effects were mild.

Compared to other studies, the present investigation shows a high response rate, ie, 50%, in patients with Hodgkin's disease. Hellriegel (2), reviewing seven phase II studies of vindesine in Hodgkin's disease, reported

TABLE 1.—Results of treatment with vindesine*

Disease	No. of patients	Response			
		CR	PR	SD	PD
Hodgkin's disease	26	1	12	8	5
Lymphoma	24	2	4	8	10
Breast cancer	24	0	4	10	10

*CR = complete response, PR = partial response, SD = stable disease, and PD = progressive disease.

an 18% PR rate among 39 patients, while Young et al (3) reported three PRs in 26 patients. Hellriegel (2) reported a 30% response (CR + PR) to vindesine in 67 collected cases of lymphoma, compared to our 25% rate. In breast cancer, Nagel (4) reported a 26% response to vindesine in 166 patients, whereas we achieved a 16.6% rate, with no CR.

We conclude from the present study that vindesine has a role in the management of refractory or relapsing Hodgkin's disease and lymphoma, and to a lesser extent, breast cancer.

REFERENCES

1. Dyke RW, Nelson RL, and Brade WP. Vindesine. A short review of preclinical and first clinical data. Cancer Chemother Pharmacol 2:229–232, 1979.
2. Hellriegel KP. Treatment of leukemias and malignant lymphomas with Vinca Alkaloids, with special reference to vindesine—a critical review. *In* Proceedings of the International Vinca Alkaloid Symposium. Vindesine. Frankfurt, 1980, pp 159–170.
3. Young C, Gralla R, and Tan C. Phase II evaluation of vindesine in patients with advanced neoplastic disease. *In* Proceedings of the Sixth Vinca Alkaloid Symposium. Vindesine. London, 1979, pp 75–83.
4. Nagel GA. Therapeutic results with vinca alkaloids in metastatic breast cancer, with special reference to vindesine. A critical review. *In* Proceedings of the International Vinca Alkaloid Symposium. Vindesine. Frankfurt, 1980, pp 214–220.

Nazli Gad-El-Mawla
M. R. Hamza
M. El-Serafi
A. El-Khoudary
M. Aboul-Einein
S. El-Demerdash

Departments of Medical Oncology and Clinical Pathology
National Cancer Institute
Fom-El-Khaleeg
Cairo, Egypt

November 3, 1982

Raynaud's Phenomenon and Cryoglobulinemia During Chemotherapy for Testicular Carcinoma

Although uncommon, Raynaud's phenomenon may be associated with neoplastic disease (1), as well as obliterative arterial disease, connective tissue disorders such as scleroderma, or use of ergot derivatives. Recently, a causal relationship between Raynaud's phenomenon and the anticancer drug bleomycin has been suggested (2); the following case report might be of interest.

CASE REPORT

The patient, an unemployed, white 18-year-old, had a right inguinal orchiectomy for an undifferentiated testicular teratoma in August 1980. Lymphography revealed widespread para-aortic nodal involvement. Preoperative plasma levels of alpha-fetoprotein (AFP) and β-subunit human chorionic gonadotropin (β-HCG) were not available but were 66 μg/L and 1450 IU/L, respectively, 7 days postoperatively. Systemic treatment was started in September 1980, using the triple drug protocol PVB [described by Einhorn (3), comprising cisplatin, vinblastine, and bleomycin]. A good response occurred, with complete regression of abdominal nodes and a return to normal levels of AFP and β-HCG. Reassessment included a computerized tomographic body scan and laparotomy in May 1981 and confirmed the complete remission.

From February 1981, the patient increasingly complained of numbness, tingling, and discoloration in the fingertips of the left hand, which turned pale and then blue in cold weather and "burned" on warming. Even after stopping chemotherapy in March, cyanosis in the fingertips persisted. Investigations included a search for rheumatoid factor, antinuclear factor, LE cells, and cold agglutinins (all negative); the erythrocyte sedimentation rate was 12, and immunoglobulins were normal, with positive IgG and IgA cryoglobulins of 17.0 and 1.0 g/L, respectively. Platelets were found to be hypersensitive, with a low platelet aggregation ratio (PAR) of 0.5 (normal range, 0.75–1.2).

In September, 6 months after stopping chemotherapy, when early atrophic changes were present at the fingertips of the left hand, persistent hypersensitive platelet aggregation was again seen with a PAR of 0.25. However, cryoglobulins were not found and all antibody tests were again negative. Treatment with aspirin and dipyridamole (Persantine) was started, with minimal improvement.

The patient remained in complete remission from the testicular teratoma, but signs of digital ischemia, with resorption of the tip of the terminal phalanx of one finger seen on X-ray, persisted 18 months after chemotherapy was stopped and 2 years from diagnosis.

DISCUSSION

PVB has been established as first-line cytotoxic therapy for advanced teratoma, but its increasing use has

not been without significant toxicity. An associated Raynaud's phenomenon has been reported (2) with this drug regimen, and a recent large study (4) suggested that it may be due to a synergistic toxicity of vinblastine and bleomycin. Raynaud's phenomenon has, however, been reported with bleomycin used alone (5), and this appears to be the most likely causative agent.

In all the reported cases, signs of digital arterial damage developed when the malignant disease was in regression; in no cases were cryoglobulins or hypersensitive platelets found. Although the hypersensitive platelet function persisted, the finding of positive IgG and IgA cryoglobulins on this one occasion could not be repeated, and it may not be significant. However, it might offer a possible mechanism for small vessel occlusion, and certainly cryoglobulins deserve some attention for their possible role in some of the other side effects of bleomycin, including the pulmonary changes. With the increasing incidence of this disease in England and Wales (6), and presumably elsewhere, and thus with the ever more frequent use of these cytotoxic agents, no doubt the true importance of cryoglobulinemia will emerge.

REFERENCES

1. PALMER HM, and VEDI KK. Digital ischaemia and malignant disease. Practitioner 213:819–822, 1974.
2. TEUTSCH C, LIPTON A, and HARVEY HA. Raynaud's phenomenon as a side effect of chemotherapy with vinblastine and bleomycin for testicular carcinoma. Cancer Treat Rep 61:925–926, 1977.
3. EINHORN LH. *Cis*-diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293–298, 1977.
4. VOGELZANG NJ, BOSL GJ, and JOHNSON K. Raynaud's phenomenon: a common toxicity after combination chemotherapy for testicular cancer. Ann Intern Med 95:288–292, 1981.
5. SUNSTRUP B. Raynaud's phenomenon after bleomycin treatment. Med J Aust 266, 1978.
6. DAVIES JM. Testicular cancer in England and Wales: some epidemiological aspects. Lancet 1:928–931, 1981.

Peter R. Dunlop*
Peter M. Hendy-Ibbs

Department of Radiotherapy and Oncology
Weston Park Hospital
Sheffield S10 2SJ
England

November 17, 1982

**Reprint requests to:* Dr. Peter R. Dunlop, Flat 10, Testwood, Golden Manor, Hanwell W7, England.

Dantrolene and Potentially Fatal Hyperthermia Secondary to L-Asparaginase

Among the potential toxic effects of L-asparaginase, the Physicians' Desk Reference (1982 edition) lists "fatal hyperthermia." Our review (1,2) of the literature and communication with the manufacturer failed to reveal any documented cases of hyperthermia, although some patients were febrile in the ordinary sense secondary to malignancy or infection. We report here a patient with extreme hyperthermia which resolved after the use of dantrolene (Dantrium, Norwich-Eaton, Norwich, NY).

CASE REPORT

A 13-year-old white male had a diagnosis of acute lymphocytic leukemia in November 1980. Remission was induced with vincristine, prednisone, and L-asparaginase and was maintained with 6-mercaptopurine, methotrexate, cytarabine, cyclophosphamide, and doxorubicin. Testicular relapse occurred in April 1982 and was treated with radiation. Bone marrow relapse occurred on July 13, 1982. The patient began to receive allopurinol and he was given methotrexate (140 mg iv) on July 14 and *Escherichia coli* L-asparaginase (Elspar, Merck, Sharp and Dohme, West Point, PA; 20,000 units im) on July 15. Three hours after receiving the L-asparaginase, he returned to his physician with a temperature of 105.6°F. There were no signs of infection before or after the injection of L-asparaginase. He was admitted to the hospital where his temperature continued to increase to a maximum of 108.6°F. With the use of ice packs, the temperature decreased to 106.8°F. At that point he received dantrolene (100 mg iv over 1 hour [1.7 mg/kg]). At the beginning of the infusion the temperature was 105.8°F and after the infusion it was 103°F. It continued to drop over the next hour to 101°F and within 4 hours of the infusion it was normal. The patient remained afebrile and was dismissed from the hospital the next day. Serum creatinine phosphokinase drawn the following morning was 7 units/L (normal range, 0–83 units/L). Blood cultures were negative and no clinical signs of infection developed subsequently. There was no recurrence of hyperthermia. The patient continued

to receive methotrexate but did not receive L-asparaginase again. He died on August 11, 1982, of progressive leukemia.

Although the Physicians' Desk Reference lists fatal hyperthermia as a possible toxic effect of L-asparaginase, we are unaware of any other documented cases of its actual occurrence. Dantrolene is effective in treating anesthesia-related malignant hyperthermia by its specific action in depressing calcium release from sarcoplasmic reticulum (3) as well as in the treatment of heat stroke (4–6). The rapid decrease in body temperature following administration of dantrolene to our patient should lead to consideration of its use under similar circumstances, because fever increases the metabolic rate and thus the heat production of skeletal muscle nonspecifically and dantrolene will rapidly halt that.

REFERENCES

1. NESBIT ME, ENTEL I, and HAMMONS GD. L-Asparaginase as a single agent in acute lymphocytic leukemia: survey of studies from Childrens Cancer Study Group. Cancer Treat Rep 65 (suppl 4):101–107, 1981.
2. OHNUMA T, ROSNER F, LEVY RN, ET AL. Treatment of adult leukemia with L-asparaginase (NSC-109229). Cancer Chemother Rep 55:269–275, 1971.
3. GRONERT GA, THOMPSON RL, and ONOFRIO BM. Human malignant hyperthermia: awake episodes and correction by dantrolene. Anesth Analg 59:377–378, 1980.
4. LYDIATT JS, and HILL GE. Treatment of heat stroke with dantrolene. JAMA 246:41, 1981.
5. DENBOROUGH MA. Heat stroke and malignant hyperpyrexia. Med J Aust 1:204–205, 1982.
6. CHABOT P de R, ELKHARRAT D, CONSO F, ET AL. Syndrome malin des neurolystiques. Nouv Presse Med 11:1067–1069, 1982.

William A. Smithson

Department of Pediatrics

Gerald A. Gronert

Department of Anesthesiology

Mayo Clinic
Rochester, MN 55905

Kenton K. Moss

Kossuth Family Health Center
Algona, IA

November 17, 1982

Effect of Intermittent and Continuous Administration of L-Cysteine Hydrochloride on L1210 Mouse Leukemia[1]

Cell culture studies in normal and malignant cell lines have demonstrated that the leukemic cells require preformed L-cysteine while normal cell lines do not (1,2). The greater incorporation of ^{35}S-L-cystine compared to control cells was attributed to decreased activity of γ-cystathionase (3) in the leukemic cells. This study was done to determine the effect(s) of increasing the intracellular concentration of L-cysteine in leukemic leukocytes by the continuous loading of L-cysteine in vivo.

Female DBA/2 mice (Jackson Labs, Bar Harbor, ME), with an average weight of 19.1 g and approximately 10 weeks old, were used in the study. Two passages of the L1210 cell line preceded the use of the cell line.

A range of concentrations of L-cysteine hydrochloride monohydrate (Sigma Chemical Co, St. Louis, MO) were prepared for administration of total daily doses of 0, 25, 100, or 400 mg/kg of body weight divided into 0.1-ml injections given ip every 3 hours for 142 hours. The solutions were adjusted to a pH of 6.0 ± 0.1 with NaOH and the osmolality to 315 ± 10 mosmols/kg·H_2O with NaCl. An acidic solution is necessary to prevent the oxidation of L-cysteine to L-cystine, and to prevent the latter from precipitating out of solution. The solutions were then passed through 0.22-μm Millipore filters into 30-ml sterile dosette vials. This regimen was decided upon due to the short plasma half-life of L-cysteine following ip injection in rats, which is approximately 2 hours. Each dose was administered to ten mice in each group of 40 mice.

In a second study, a solution of L-cysteine HCl-H_2O was prepared at a concentration of 373 mg/ml. This was accomplished by alternately saturating deoxygenated H_2O under N_2 with the free base of L-cysteine and then adding L-cysteine HCl H_2O until a pH of 2.0 was reached at 25°C. The solution was then passed through a 0.45-μm Millipore filter, loaded into 12 model 2002 Alzet osmotic minipumps (Alza, Palo Alto, CA), and placed in sterile saline. Eight control minipumps containing an

[1]Supported in part by Public Health Service grant CA-22170 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, by IN-125c from the American Cancer Society, and by the Hematology-Oncology Research Fund of the Medical College of Pennsylvania.

HCl solution at a pH of 2.0 were also prepared similarly and implanted ip.

The osmotic minipumps require approximately 4 hours of exposure to an aqueous medium before they begin to deliver their contents at the rate of 0.88 μl/hour. The length of the implantation procedure for all of the mice was just under 4 hours. The six mice receiving L-cysteine received 750 mg/kg/day of L-cysteine HCl H_2O. Wbc counts were taken and smears were made daily for 6 days.

The survival of DBA/2 mice bearing L1210 leukemia cells demonstrated a significant prolongation of survival of 165 ± 5 hours ($P < 0.05$) in the group treated with 400 mg/kg/day of L-cysteine HCl compared to 150 ± 1 hours in the control group. The total daily doses of L-cysteine HCl of 25 or 100 mg/kg/day did not alter the survival of the L1210-bearing mice. In this experiment, L-cysteine HCl was administered ip every 3 hours, for a total of 142 hours.

The effects of the multiple ip injections of L-cysteine HCl administered every 3 hours caused a marked body weight loss in control DBA/2 mice without L1210 cells. The control group of mice lost an average of 2 g in 144 hours after a total of 48 ip injections/mice. An initial weight loss in the L1210-bearing mice given the same number of ip injections was observed, but the weight gain at 96 and 120 hours was attributed to the accumulation of ascitic fluid caused by the leukemic cells in the peritoneal cavity.

There were no significant differences in the daily wbc counts in the cysteine-treated or the control mice in the second study. Microscopic examination of the smears showed a similar percentage of leukemic cells in the groups with or without minipumps containing cysteine or HCl acid. The peripheral blast cells comprised 10% of the circulating leukocytes and appeared in the circulation between 120 and 140 hours after transplantation. There was no difference in the survival of mice in the three groups.

In conclusion, the administration of high doses of L-cysteine did not affect the rapid proliferation of L1210 lymphoblasts in DBA/2 mice. We have previously demonstrated low taurine levels in the L1210 leukemic cells (4,5), which is the end product of the methionine-cysteine biochemical pathway. Administration of high concentrations of taurine in L1210-bearing mice did not alter the survival curve in our previous experiments, despite a high intracellular uptake by the leukemic cells similar to the findings in this study. These observations indicate that the sulfur-containing amino acid pathway may be secondarily affected by the abnormal proliferation of the leukemic cells. The reduced cystathionase activity may be the result of a low concentration of this enzyme rather than an inhibition. The rapid proliferation of the leukemic leukocytes may cause a depleted metabolite pool, which may affect the transcription for this enzyme or the synthesis of it.

REFERENCES

1. Uren JR, and Lazarus H. L-Cyst(e)ine requirements of malignant cells and progress toward depletion therapy. Cancer Treat Rep 63:1073–1079, 1979.
2. Iglehart JD, York RM, Modest AP, et al. Cystine requirement of continuous human lymphoid cell lines of normal and leukemic origin. J Biol Chem 252:7184–7191, 1977.
3. Glode LM, Greene HL, and Bikel I. γ-Cystathionase in normal and leukemic cells. Cancer Treat Rep 63:1081–1088, 1979.
4. Baskin SI, Wakayama K, Knight T, et al. The sulfur-containing amino acid pathway in normal and malignant cell growth Taurine. *In* Nutrition and Neurology (Huxtable RJ, and Pasantes Morales H, eds). Plenum Publishing Co, 1982, pp 127–141.
5. Wakayama K, Besa EC, Jepson JH, et al. Cell volume and chemical analysis of L1210 cells. Exp Hematol 7:70, 1979.

Thomas Knight[2]
Emmanuel C. Besa*
Rosaline R. Joseph
Steven I. Baskin[3]

Departments of Medicine (Section of Hematology and Oncology) and Pharamacology
The Medical College of Pennsylvania
3300 Henry Ave
Philadelphia, PA 19129

November 24, 1982

[2]*Present address:* The Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey.

[3]We wish to thank Dr. Mary B. Fields for her continued encouragement, Edward K. Wikoff for his assistance and laboratory skills, and Mrs. Charlene Flamer and Ms. Donna M. Scharff for preparing the manuscript.

*To whom reprint requests should be sent.

Amsacrine (AMSA) in the Treatment of Lymphoma

The recent article by Micetich et al (1) is somewhat misleading in that it contains a statement that amsacrine (AMSA) produces "known significant response rates" in lymphoma. The best results using AMSA in lymphoma were reported in a preliminary communication by Cabanillas et al (2) who noted six major re-

sponses in a series of 30 patients with advanced disease. However, a recent update of that study showed only one additional response in the next 20 patients entered, for an overall response rate of 14% (seven of 50 patients) (3). Moreover, substitution of AMSA for methotrexate in a salvage protocol for patients with early relapse (IMVP-16 vs AIVP-16) produced a significantly inferior rate of response in patients who received the combination containing AMSA (4).

Previously, we had observed no major responses in 21 patients with non-Hodgkin's lymphoma treated with AMSA (5). Moreover, the Eastern Cooperative Oncology Group has recently concluded a larger study with similarly negative results in non-Hodgkin's lymphoma (A. Keller, personal communication). The failure to observe any major responses in seven patients treated at close to the maximally tolerated (phase II) dose in the Micetich study is likewise not encouraging.

Proper case selection is always a major factor in the interpretation of the results of phase II studies. However, based on currently available data, AMSA does not appear to have major antitumor activity in patients with advanced lymphoma. The usefulness of AMSA in any disorders other than the acute leukemias remains to be established.

REFERENCES

1. Micetich KC, Zwelling LA, Gormley P, et al. Phase I–II study of *m*-AMSA administered as a continuous infusion. Cancer Treat Rep 66:1813–1817, 1982.
2. Cabanillas F, Legha SS, Bodey GP, et al. Initial experience with AMSA as a single agent treatment against malignant lymphoproliferative disorders. Blood 57:614–616, 1981.
3. Cabanillas F. Results with amsacrine in the treatment of refractory lymphomas. *In* Amsacrine: Current Perspectives and Clinical Results With a New Anticancer Agent. Proceedings of an International Symposium on AMSA, Nice, France, Apr 3, 1982.
4. Cabanillas F, Hagmeister FB, Salvador P, et al. Factors predicting for complete remission (CR) in patients (pts) with recurrent lymphoma treated with salvage combination chemotherapy. Proc ASCO 1:167, 1982.
5. Warrell RP, Jr, Straus DJ, and Young CW. Phase II trial of 4'-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) in the treatment of advanced non-Hodgkin's lymphoma. Cancer Treat Rep 64:1157–1158, 1980.

Raymond P. Warrell, Jr

Developmental Chemotherapy Service
Memorial Sloan-Kettering Cancer Center
1275 York Ave
New York, NY 10021

November 30, 1982

International Symposium of Biological and Clinical Aspects of Soft and Hard Tissue Repair

Innisbrook at Tarpon Springs, Florida, May 11–15, 1983

This symposium meets the criteria for 20 credit hours in AMA Category I of the Physician's Recognition Award. Tuition is $225 and the deadline for registration is April 8, 1983. Early registration is advised because enrollment is limited.

For further information, contact: Symposia Medicus, 2880 Shadelands Dr, Suite 404, Walnut Creek, CA 94598 (telephone 415-935-7889).

Thirteenth Annual Neurosurgery Postgraduate Course and the Seventh Annual Neurosurgical Nurses' Conference

San Francisco, California, May 26–27, 1983

Presented and sponsored by the University of California School of Medicine's Department of Neurological Surgery and Extended Programs in Medical Education, this course will provide an update on selected topics in clinical neurosurgery.

Cerebral tumors, spinal injuries and lesions, cerebrovascular disease, and recent clinically relevant advances in the neurologic sciences will be covered in depth. In addition to the didactic presentations, small luncheon discussion sessions will give participants an opportunity to interact with faculty. This program meets the criteria for 15 AMA/CMA Category 1 credit hours.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143 (telephone 415-666-4251).

Taste and Smell

New York, New York, June 2–3, 1983

A conference on taste and smell in humans will be held at Memorial Sloan-Kettering Cancer Center. Clinicians and basic scientists will take part in an exchange which is aimed at improving and increasing the clinical diagnostic evaluation of taste and smell.

For further information, contact: Dr. Richard S. Rivlin, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone 212-794-7703).

Diet, Nutrition, and Cancer: Etiologic and Treatment Issues

Boston, Massachusetts, June 2–4, 1983

The Cancer Committee of the New England Deaconess Hospital is sponsoring its twelfth annual cancer course to be held at the hospital in Boston.

For more information contact: Department of Continuing Education, Harvard Medical School, 25 Shattuck St, Boston, MA 02115 (telephone: 617-732-1525).

Predictive Drug Testing on Human Tumor Cells

Zurich, Switzerland, July 20–22, 1983

An international conference on predictive drug testing on human tumor cells will be held at the University Hospital of Zurich. The conference will deal with newly developed techniques for individualizing treatment of cancer patients. The program will include lectures by invited speakers, competitive papers, and a poster session. The deadline for receipt of abstracts is April 15th, 1983.

For further information, contact: V. Hofmann, MD, Division of Oncology, University Hospital, 8091 Zurich, Switzerland.

Gynecologic Laser Surgery

Maui, Hawaii, July 16–23, 1983

This continuing education program offering meets the criteria for AMA category 1 credit for physicians and the California and Nevada Boards of Registered Nursing for nurses.

Tuition is $285 for physicians and $230 for nurses.

For more information, contact: Symposia Medicus, 2880 Shadelands Dr, Suite 404, Walnut Creek, CA 94598 (telephone 415-935-7889).

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

International Conference on Head and Neck Cancer

Baltimore, Maryland, July 22–27, 1984

The American Society for Head and Neck Surgery and The Society of Head and Neck Surgeons are sponsoring this conference to be held at the Hyatt Regency Hotel in Baltimore's Inner Harbor. Topics will include basic research in virology, biochemistry, immunology, cell and tissue culture and other areas; etiologic factors; carcinogens; viral studies; epidemiology; early detection; diagnosis and staging; results of surgery, radiation therapy and chemotherapy; modifications of standard treatments; new treatment modalities; nutrition; rehabilitation; reconstruction; design of clinical trials; and statistical considerations.

The conference will provide dissemination of information through didactic sessions, symposia, panels, workshops, cine discussions, and scientific exhibits. Abstracts are invited for oral presentations and scientific exhibits.

For further information, contact: The Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Baltimore, MD 21201 (telephone 301-528-3956).

Position Vacancy: Associate Director, Developmental Therapeutics Program

The position of *Associate Director of the Developmental Therapeutics Program* is vacant. This position is located within the Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health, Public Health Service, Department of Health and Human Services.

The NCI develops the overall national strategy against cancer, coordinates federal government and non-federal government cancer programs, and supports international programs of cancer research. The Division of Cancer Treatment directs the NCI's major research effort related to the treatment of cancer. The Developmental Therapeutics Program plans, directs, and conducts a basic applied research program in the preclinical development of chemotherapeutic agents. This Program is comprised of in-house laboratories as well as several branches which administer a 75-million dollar/year grant and contract program through a staff of 275.

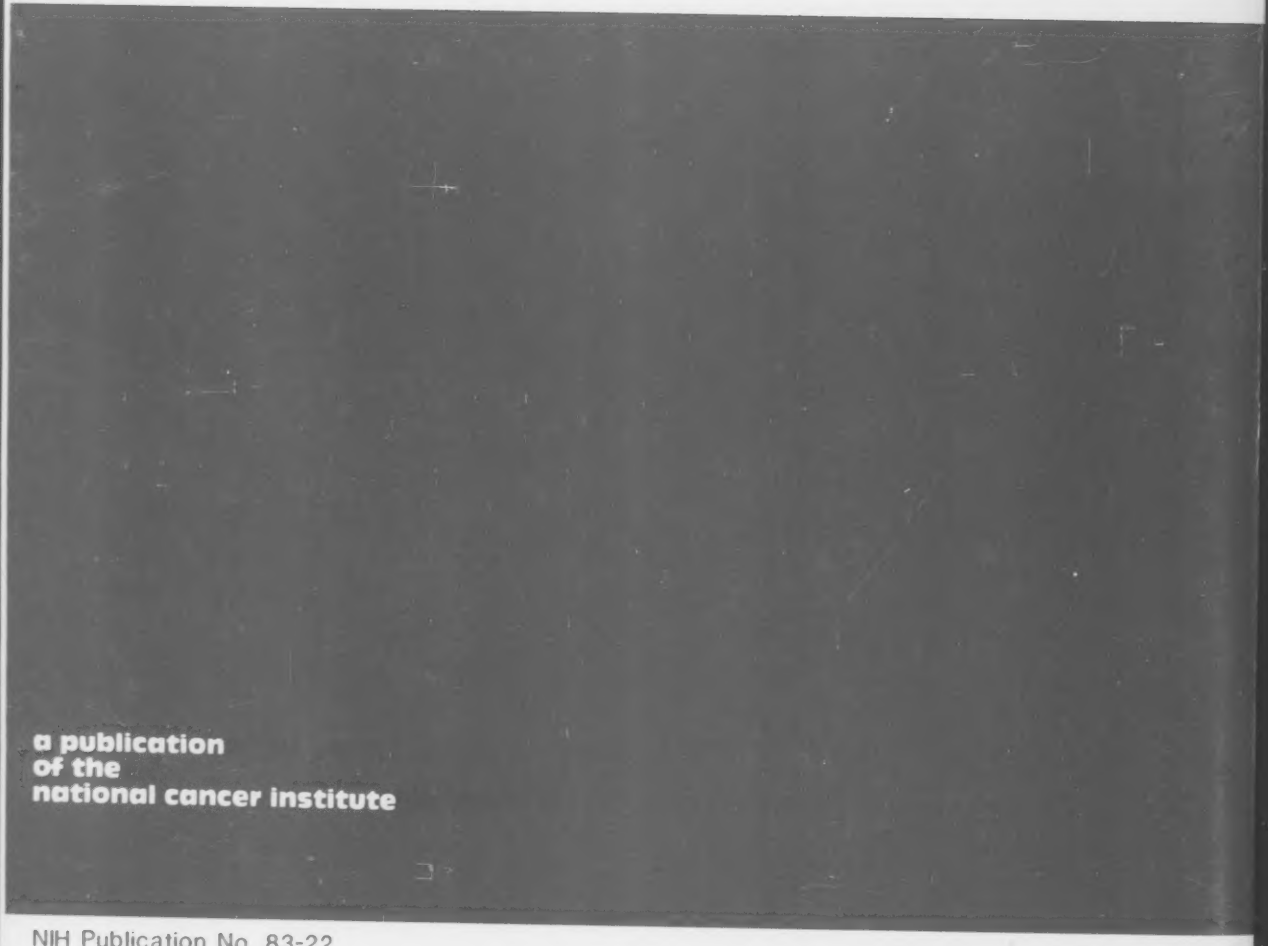
This is a Civil Service position in the Senior Executive Service (SES) with a salary range from $56,945 to $63,800 per annum. Candidates may be eligible for Physician's Comparability Allowance up to $10,000 per year. (Alternatively, candidates may be eligible for appointment in the Commissioned Corps of the U. S. Public Health Service.) The individual selected, if not presently in SES, must serve a 1-year probationary period.

Candidates must meet established mandatory professional/technical and managerial/executive requirements. Candidates will be further evaluated on the extent they exceed the minimum requirements and on additional factors including scientific reputation/peer recognition, training, and education and honors and awards. A copy of the requirements may be obtained by contacting Mrs. Sara Benesch, Personnel Management Branch, NCI (301) 496-6862.

Applications (Personal Qualifications Statement SF-171) are to be sent to Mrs. Benesch at: Personnel Management Branch, National Cancer Institute, Bldg 31, Rm 3A34, National Institutes of Health, Bethesda, Maryland 20205. A current curriculum vitae and bibliography must accompany all applications. Applications from women and/or minority group members are especially solicited.

The National Institutes of Health is an EQUAL OPPORTUNITY EMPLOYER.

☆U.S. GOVERNMENT PRINTING OFFICE: 1983 381 140 9



NIH Publication No. 83-22